NIH - University of Oxford (England)

National Institutes of Health Investigator:

Robert Balaban

rsb@zeus.nhlbi.nih.gov

University Professor:

Kieran Clarke

Kieran.Clarke@bioch.ox.ac.uk

Cardiac Energetics in Mouse Models of Friedreich Ataxia

Friedreich ataxia, the most common autosomal recessive ataxia with a frequency of 1 in 50,000 live births, is often associated with cardiomyopathy and increased incidence of diabetes.1 Cardiac dysfunction is the most frequent cause of death. Friedreich ataxia is caused by severely reduced levels of frataxin, a mitochondrial protein of unknown function. There is evidence that frataxin deficiency leads to a severe defect in mitochondrial respiration associated with abnormal mitochondrial iron accumulation.2 Skeletal muscle3 and heart4 bioenergetic defects have been found in Friedreich ataxia patients and cardiomyopathy has been reported for mouse models of Friedreich ataxia.1 Owing to the reported effects of frataxin on cellular oxidative phosphorylation,5 we propose to determine whether cardiac function and energetic defects occur in mouse models of Friedreich ataxia using magnetic resonance (MR) imaging and 31P MR spectroscopy.6 We will determine whether the mice are diabetic, whether the hearts are insulin resistant7,8 and whether defects are normalised in transgenic mice with upregulated frataxin.9 We will determine cardiac efficiency in the isolated working heart and uncoupling in isolated mitochondria.10 At the NHLBI, we will use triple photon confocal microscopy to determine mitochondrial NADH in intact cells11 and the kinetics of mVO2, NADH and light scattering will be determined during calcium activation of oxidative phosphorylation in isolated mitochondria.12,13 This study will increase our understanding of the metabolic abnormalities underlying Friedreich ataxia and may indicate a therapy.

References

1. Puccio H, Simon D, Cossee M, Criqui-Filipe P, Tiziano F, Melki J, Hindelang C, Matyas R, Rustin P, Koenig M. Mouse models for Friedreich ataxia exhibit cardiomyopathy, sensory nerve defect and Fe-S enzyme deficiency followed by intramitochondrial iron deposits. Nature Genetics 27:181-186, 2001.
2. Kaplan J. Friedreich's ataxia is a mitochondrial disorder. Proc Natl Acad Sci USA 96:10948-10949, 1999.
3. Lodi R, Cooper JM, Bradley JL, Manners D, Styles P, Taylor DJ, Schapira AHV. Deficit of in vivo mitochondrial ATP production in patients with Friedreich ataxia. Proc Natl Acad Sci USA 96:11492-11495, 1999.
4. Lodi R, Rajagopalan B, Blamire AM, Cooper JM, Davies CH, Bradley JL, Styles P, Schapira AHV. Cardiac energetics are abnormal in Friedreich ataxia patients in the absence of cardiac dysfunction and hypertrophy: An in vivo 31P magnetic resonance spectroscopy study. Cardiovasc Res 52:111-119, 2001.
5. Ristow M, Pfister MF, Yee AJ, Schubert M, Michael L, Zhang C-Y, Ueki K, Michael MD, Lowell BB, Kahn CR. Frataxin activates mitochondrial energy conversion and oxidative phosphorylation. Proc Natl Acad Sci USA 97:12239-12243, 2000.
6. Hopkins JCA, Bia BL, Crilley JG, Boehm EA, Sang AE, Tinsley JM, King LM, Radda GK, Davies KE, Clarke K. Muscular dystrophy: from gene to patient. MAGMA 11:7-9, 2000.
7. King LM, Sidell RJ, Wilding JR, Radda GK, Clarke K. Free fatty acids, but not ketone bodies, protect diabetic rat hearts during low-flow ischemia. Am J Physiol 280:H1173-H1181, 2001.
8. Sidell RJ, Cole MA, Draper NJ, Desrois M, Buckingham R, Clarke K. Thiozolidinedione treatment normalizes insulin resistance and ischemic injury in the Zucker fatty rat heart. Diabetes In press. 2002.
9. Pook MA, Al-Mahdawi S, Carroll CJ, Cossee M, Puccio H, Lawrence L, Clark P, Lowrie MB, Bradley JL, Cooper JM, Koenig M, Chamberlain S. Rescue of the Friedreich's ataxia knockout mouse by human YAC transgenesis. Neurogenetics 3:185-193, 2001.
10. Boehm EA, Jones BE, Radda GK, Veech RL, Clarke K. Increased uncoupling proteins and decreased efficiency in the palmitate-perfused hyperthyroid rat heart. Am J Physiol 280:H977-H983, 2001.
11. Combs CA, Balaban RS. Direct imaging of dehydrogenase activity within living cells using enzyme-dependent fluorescence recovery after photobleaching (ED-FRAP). Biophys J 80:2018-2028, 2001.
12. Territo PR, French SA, Dunleavy MC, Evans FJ, Balaban RS. Calcium activation of heart mitochondrial oxidative phosphorylation. J Biol Chem 276:2586-2599, 2001.
13. Territo PR, Mootha VK, French SA, Balaban RS. Ca2+ activation of heart mitochondrial oxidative phosphorylation: role of the F0/F1-ATPase. Am J Physiol 278:C423-C435, 2000.

NIH - University of Oxford (England)

National Institutes of Health Investigator:

Jay Berzofsky

jb4q@nih.gov

University Professor:

Vincenzo Cerundolo

vincenzo.cerundolo@imm.ox.ac.uk

The overall project deals with the role of NKT cells, restricted by CD1d, by using CD1d-tetramer developed by Cerundolo's laboratory, as a part of the innate immune system that interfaces with and in part regulates the adaptive immune system of T and B lymphocytes in health and disease. We are especially interested in the role of these cells in cancer and chronic viral infection such as HIV and hepatitis C virus infection.

The immune system has two arms to defend against pathogens or abnormal cells in the body. One is innate immunity and the other is adaptive immunity. Understanding the interface between innate and adaptive (acquired) immunity is of great current interest to the field of immunology because it is central to understanding the mechanisms of regulation of immune responses. Natural Killer T (NKT) cells are T cells which also express NK cell markers, and are usually restricted by the non-classical class I MHC molecule CD1d, presenting a glycolipid rather than a peptide. This cell has some features of both innate and adaptive immune cells, and recently has been shown to regulate many immune reactions which cause and prevent disease. However, since this is a very small population, it has been very difficult to study. Tetramer technology is a one of the cutting edge technologies permitting detection of a small number of T cells by using a flow cytometer. The Cerundolo laboratory at Oxford University has developed tools to identify these NKT cells and to study their interaction with the adaptive immune system of CD4+ and CD8+ T cells as well as B cells. The Oxford laboratory has also developed protocols to load CD1d tetramers with single glycolipids to identify physiologically relevant ligands recognized by these NKT cells. The Berzofsky/Terabe laboratory at NIH has discovered a novel negative regulatory pathway initiated by CD1d-restricted NKT cells that downregulates tumor immunosurveillance. The pathway involves secretion of IL-13 by the NKT cells that induces a CD11b+Gr-1+ myeloid cell to make TGF-beta, which then suppresses the induction of the CD8+ cytotoxic T lymphocytes (CTL) that mediate the tumor immunosurveillance. The cell controlling this regulatory pathway is the NKT cell, but the signals that activate it and the factors that determine whether this cell makes IL-13 and acts as a suppressor or makes interferon-gamma and facilitates the anti-tumor response are unknown. Thus, it would be of great interest to isolate the relevant glycolipids produced by the tumor cell that bind to CD1d and activate this regulatory NKT cell. The approaches developed by the Oxford component of the collaboration should make it possible to identify such CD1d ligands and determine their role in initiating the regulatory response.

Furthermore, it is important to define the role of these negative regulatory NKT cells in human cancer and chronic viral infections in which immunodeficiency is observed, such as HIV infection. The ability to stain these cells with tetramers of human CD1d loaded with different lipids, and to further characterize these cells by the limited repertoire of T cell receptors used, should facilitate the exploration of the role of these innate immune cells in regulating adaptive immune responses in human disease.

The student would work on CD1d molecules, tetramers, their lipid ligands, and their ability to prime a CD1d-restricted immune response in Oxford, on the murine cancer models at NIH, and on human NKT cells in different disease states in both Oxford and NIH.

NIH - University of Oxford (England)

National Institutes of Health Investigator:

Juan Bonifacino

juan@helix.nih.gov

University Professor:

Gillian Griffiths

Biogenesis and Function of Lysosome-related Organelles

Specialized secretory mechanisms enable proteins and peptides to be stored in subcellular organelles and released when required in response to an extracellular signal. This "regulated" secretion enables cells to control the release of active proteins. This level of control is critical for these cells to function properly. This is particularly important in cells of the immune system. Secretion is required to elicit an immune response, but needs to be directed only in response to infected cells.

The mechanisms that control secretion in immune cells are shared by melanocytes which give rise to pigmentation in skin and hair. Lymphocyte secretory granules and melanosomes belong to a family of organelles known as "lysosome-related organelles" due to their shared biogenetic pathways with lysosomes. Loss of function of any of the proteins controlling secretion in lymphocytes and melanocytes would therefore give rise to varying degrees of immunodeficiency and albinism. Hermansky Pudlak syndrome(HPS) is a genetic disease characterized by these defects. This project aims to identify the function of proteins encoded by the genes mutated in HPS in melanocytes and immune cells and thereby identify the machinery that controls secretion in these cells.

NIH - University of Oxford (England)

National Institutes of Health Investigator:

Louise Brinton

brintonl@exchange.nih.gov

University Professor:

Valerie Beral

Nicky.langston@cancer.org.uk

Hormones and Breast Cancer

There is increasing evidence that a woman's risk of developing breast cancer is increased the higher her levels of oestrogen are. Little is known about why some women have high levels of oestrogens and others do not. The main aim of the project would be to look for novel markers of high oestrogen levels, including genetic and metabolic factors and the interrelation between oestrogen levels and levels of other hormones. The research groups in Oxford and Washington have an international reputation for research into the causes of breast cancer and have access to large stores of biological materials and lifestyle data for women with breast cancer.

NIH - University of Oxford (England)

National Institutes of Health Investigator:

Susan Buchanan

skbuchan@helix.nih.gov

University Professor:

Declan Doyle

declan@biop.ox.ac.uk

Structure determination of the divalent metal ion transporter NRAMP: Understanding Host-Pathogen Interactions

When microbial pathogens invade a eukaryotic host, they are usually engulfed and then destroyed by macrophages. One microbe-destruction mechanism used by macrophages is to deprive microbes of the divalent metal ions needed for survival. NRAMPs, which are natural resistance-associated macrophage proteins, are divalent metal ion transporters found in macrophage (and other) membranes. In macrophages, NRAMP1 functions to pump iron and manganese out of the organelle, which limits the replication of the engulfed pathogen. Interestingly, NRAMP proteins are also found in prokaryotes such as Salmonella typhimurium, Mycobacterium tuberculosis, and Escherichia coli. In contrast to eukaryotic NRAMP proteins, bacteria use NRAMP proteins to pump divalent metal ions into the cell.

Although the prokaryotic and eukaryotic NRAMPs function antagonistically, both types are found in the plasma (or inner) membrane. Secondary structure predictions differ for the various NRAMP family members; they are predicted to contain 9 - 12 transmembrane alpha helices. However, prediction programs can be inaccurate and the possibility exists that all family members share a common fold. Very little is presently known about how ions are selected and transported across the membrane.

The goal of this project is to determine a high resolution structure of an NRAMP protein by X-ray crystallography. The student selecting this project will first screen prokaryotic and eukaryotic NRAMP proteins for high level expression in E. coli. The selected transporter will then be chromatographically purified and crystallised in the presence of detergent. Crystals will be analysed by X-ray diffraction techniques and the structure will be solved using isomorphous replacement or anomalous diffraction methods. Once the project is completed, the student will have gained experience in all aspects of membrane protein structural biology: from gene cloning to protein expression, purification, crystallisation, and structure determination by X-ray crystallography. Furthermore, the student will gain an understanding of the molecular basis for metal ion transport so important to both prokaryotic pathogens and their eukaryotic hosts.

Further reading:
NRAMPs

Strauss, E. (2000). Microbes display their versatility at ASM meeting. Science 288, 1958-1959.

Forbes, J.R. & Gros, P. (2001). Divalent-metal transport by NRAMP proteins at the interface of host-pathogen interactions. Trends Microbiol. 9, 397-403.

Cuellar-Mata, P., Jabado, N., Liu, J., Furuya, W., Finlay, B.B., Gros, P. & Grinstein, S. (2002). Nramp1 modifies the fusion of Salmonella typhimurium-containing vacuoles with cellular endomembranes in macrophages. J. Biol. Chem. 277, 2258-2265.

Membrane protein crystal structures

Doyle, D.A., Cabral, J.M., Pfeutzner, R.A., Kuo, A., Gulbis, J.M., Cohen, S.L., Chait, B.T. & MacKinnon, R. (1998). The structure of the potassium channel: molecular basis
of K+ conduction and selectivity. Science 280, 69-77.

Buchanan, S. K., Smith, B. S., Venkatramani, L., Xia, D., Palnitkar, M., Chakraborty, R., van der Helm, D. & Deisenhofer, J. (1999). Crystal structure of the outer membrane active transporter FepA from Escherichia coli. Nature Struc. Biol. 6, 56-63.

NIH - University of Oxford (England)

National Institutes of Health Investigator:

Robert Crouch

robert_crouch@nih.gov

University Professor:

Ed Southern

Cellular, Viral, and Therapeutic Roles of RNA-DNA Hybrids

Many diseases are the result of failure to replicate or repair damaged DNA. The maintenance of DNA integrity and sequence is critical for cell survival and propagation. RNA-DNA hybrids are essential intermediates in DNA replication and repair in cells and in retroviruses such as HIV. Following their use as primers for DNA synthesis, RNA-DNA hybrids are normally removed by ribonucleases (RNases H) that are specific for RNA in RNA-DNA duplexes. For HIV-1, RNase H activity is an integral part of the enzyme converting the viral RNA into double-stranded DNA prior to integration of the DNA into host's chromosomal DNA. Inactivation of the RNase H renders the virus non-infectious, making this enzymatic activity a target for therapy of this dreaded disease. The structure and enzymatic cleavage mechanism are similar for both viral and cellular proteins making it important to either target the drug specifically to the HIV protein or make certain that inactivation of the cellular enzyme does not lead to unacceptable side effects.

Most organisms have multiple forms of RNase H. Two RNases H that are dissimilar in primary amino acid sequence but have similar structural and, presumably, a common enzymatic mechanism. To address the cellular roles of these RNases H, we have examined the effects of eliminating RNases H in unicellular organisms such as bacteria and fungi. Prokaryotic and eukaryotic cells deleted for either or both RNase H-encoding genes grow quite well but with some important differences from the parental cells. These results suggest that drugs targeted to HIV RNase H need to be examined to see if they provoke effects similar to those found in RNase H-deletion strains (e.g., increased sensitivity to DNA-damaging agents). Examinations of RNase H in mouse and human cells is currently a major focus of our research.

Another important type of RNA-DNA hybrid is that formed by DNA introduced for specific disease treatment. Drug development employing therapeutic DNA oligonucleotides (TOs) is based on the endogenous RNases H for activity. In many diseases, a protein is made at an inappropriate time or location. Elimination of synthesis of the protein would lessen or eliminate the disease. For example, expression of protein X in heart tissue, an organ in which protein X is usually absent, would lead to major defects in the heart. The messenger RNA for protein X carries the information that specifies the amino acid sequence for the heart tissue to make protein X. The messenger RNA encoding the undesired protein X would be the target for a TO complementary to the mRNA thereby generating a substrate recognized by RNase H initiating degradation of the RNA. Some important features of action of TOs we are examining are: Do both cellular RNases H act to destroy RNAs targeted by TOs? Can the RNase H be modulated in the cell to maximize or in some cases minimize the effectiveness of the TOs? Our experiments using TOs are being carried out in collaboration with Drs. E. Southern and M. Sohail of Oxford University.

NIH - University of Oxford (England)

National Institutes of Health Investigator:

Marinos Dalakas

dalakasm@ninds.nih.gov

University Professor:

Angela Vincent

Molecular Targets for Pathogenic Antibodies in the Stiff Person Syndrome

Stiff person syndrome (SPS) is a disabling central nervous system disorder characterised by muscle rigidity with painful spasms, probably caused by reduced function of the spinal interneurons that control reciprocal muscle activity. It is frequently associated with diabetes and other autoimmune disorders, and has recently been shown by the NIH group to respond well to intravenous immunoglobulin therapy, a well-established treatment for autoimmune disorders (Dalakas et al New Eng J Med 345: 1870-6, 2001). Antibodies to glutamic acid decarboxylase (GAD) are present in over 60% of patients, but it is not clear whether these antibodies cause the loss of spinal inhibition. Indeed, since GAD is an intracellular enzyme, it seems more likely that the causative antibody is directed against the extracellular aspect of a cell membrane target, as is the case in all of the well-established antibody-mediated neurological disorders (reviewed in Vincent et al J Neuroimm 100: 169-180, 1999).

The project will be to define new, potentially pathogenic, neuronal antibodies and to explore their mechanisms of action. Serum IgG from patients will be studied for binding to cultured cell lines and primary cultures of spinal cord cells using the fluorescent activated cell sorter (Oxford). The effects of SPS IgG on the function and viability of the neuronal cultures will be tested, and the immunolocalization of IgG will be examined with confocal microscopy and immuno-electronmicroscopy (Oxford/NIH). These approaches should confirm that there are serum antibodies that bind to and affect the function of appropriate neuronal cell populations, but will not define the antigenic target(s). On the basis of the FACS data with different cell lines, however, it will be possible to use subtractive hybridisation, microarrays and human genome searches to identify candidate antigens (Oxford). Alternatively, immunoprecipitation and 2D gel electrophoresis with SELDI mass spectrometry can be used to isolate and sequence proteins bound by the antibodies (NIH).

Once candidate antigens are defined, they can be expressed in eukaryotic cell lines for binding and functional studies (Oxford), and their localisation and expression examined in normal rodent or human (and possibly postmortem) spinal cord tissue (Oxford/NIH). Animals can be immunised against the target antigens in order to produce a model for examining in vivo effects and therapies. It will be important to test larger patient cohorts for the antibodies, and to examine their levels during treatment protocols, such as intravenous immunoglobulin and other immunosuppressive therapies (NIH).

All of these approaches are currently in use or being established in NIH and Oxford. Some, for instance, have been used in a series of studies which identified the receptor tyrosine kinase MuSK as a major new antigen in patients with myasthenia gravis who do not have acetylcholine receptor antibodies (Hoch et al Nat Med 7: 365-8; 2001). The project will provide excellent training in immunology, molecular biology and neurobiology, and should lead to real advances in the diagnosis, management and understanding of a rare but fascinating neurological disease.

NIH - University of Oxford (England)

National Institutes of Health Investigator:

Daniel Douek

ddouek@mail.nih.gov

University Professor:

Andrew McMichael

andrew.mcmichael@ndm.ox.ac.uk

T cell responses in humans with HIV vaccines

Recent phase I trials of two HIV vaccines based on A clade HIV gag are in progress in Oxford. The DNA vaccine comprises A clade gag p24 and p17, the MVA vaccine is a recombinant modified vaccinia Akara virus. Both have been shown to induce good CD8+ T cell responses after one or two injections. The combination, DNA prime and MVA boost is expected to be potent in stimulating even greater T cell responses, based on animal studies. This project will analyse these human responses in great detail using methods developed in Dr Douek's laboratory to analyse the T cell responses together with tetramer based techniques developed in Oxford. Using these methods the project will explore the generation of memory and effector CD4+ and CD8+ T cells in vaccine recipients. The responses will be compared to those in HLA matched HIV infected persons where details of the T cell responses are known, but are in part defective.

NIH - University of Oxford (England)

National Institutes of Health Investigator:

Joshua Farber

joshua_farber@nih.gov

University Professor:

David Jackson

djackson@enterprise.molbiol.ox.ac.uk

The Roles of Hyaluronan and Chemokines in Lymphocyte Trafficking Through the Lymphatic System

The lymphatic vasculature is an integral part of the immune system, comprising a vast network of vessels whose primary function is to channel leukocytes to and from the lymph nodes where they meet and present foreign antigen to naïve T and B cells. In addition, the lymphatics are a major route for early tumour metastasis in many cancers such as that of the breast, where axillary lymph node involvement is an important prognostic indicator. Yet despite their importance, we know very little about lymphatics, how lymph vessel endothelial cells differ from those of blood vessels, or how the entry and migration of leukocytes and tumour cells is regulated.

We are particularly interested in how chemokines and cell adhesion molecules may contribute to leukocyte recruitment and trafficking through lymphatics. Chemokines are cytokines that direct leukocyte migration, a small number of which are thought to be released by lymphatic endothelial cells in order to promote leukocyte trafficking to lymph nodes. Of the adhesion molecules, those that bind the extracellular mucopolysaccharide hyaluronan are likely candidates for regulating cellular trafficking through lymphatics. Hyaluronan is itself a component of lymph and can induce chemokine release. Furthermore, one of the main receptors for hyaluronan, a protein termed LYVE-1, is expressed predominantly by lymphatic endothelial cells. The project we envisage would focus on a systematic identification of the chemokines produced by lymphatic endothelium, how their synthesis and release might be induced by hyaluronan and LYVE-1 ligation, and what roles chemokines and hyaluronan might play in the recruitment of lymphocytes into lymphatics and lymph nodes draining tumours and sites of inflammation. The project will depend on studies of isolated lymphatic endothelial cells, knockout mice, and human cancers.

NIH - University of Oxford (England)

National Institutes of Health Investigator:

Gary Felsenfeld

gary.felsenfeld@nih.gov

University Professor:

Doug Higgs

drhiggs@molbiol.ox.ac.uk

MRC Molecular Haematology Unit

The Molecular Haematology Unit (MHU) is located in the Weatherall Institute of Molecular Medicine. It includes eight research teams with a total of about seventy scientists who share a common interest in understanding how the erythroid (red cell) and megakaryocytic (platelet) lineages are specified from a common haemopoietic stem cell during embryonic, fetal and adult life. We are pursuing this question with the aim of understanding the mechanisms underlying inherited (e.g. thalassaemia and congenital dyserythropoetic anaemia) and acquired (e.g. myelodysplasia and leukaemia) human genetic diseases that perturb the normal process of haemopoiesis.

In one approach, we are isolating haemopoietic progenitors at specific stages of the differentiation pathway from normal and abnormal cells. The transcriptional profiles of these purified cells can be determined by analysis on microarrays to ask which genes are switched on or off during normal haemopoiesis and leukaemogenesis. Other MHU groups are studying the roles of particular key transcriptional regulators (e.g. GATA-1, Fog-1. SCL/Tal-1 and c-Myb) of haemopoieyic differentiation. In addition, a major interest in the MHU is to understand the mechanisms by which individual genes (e.g. alpha and beta globin) are expressed in a tissue- and developmental stage specific manner during erythropoiesis. In addition to helping us understand erythropoiesis this provides a model for understanding the general mechanisms by which all eukaryotic genes are regulated.

A major impetus at present is to understand the epigenetic control (histone modification, replication timing, nuclear position, DNA methylation) of globin gene expression during haemopoiesis. In Oxford this work is being pursued in the human and mouse systems whereas at NIH the Felsenfeld laboratory is studying these issues in the chicken haemopoietic system.

Further enquiries concerning the details of specific projects can be obtained from drhiggs@molbiol.ox.ac.uk in Oxford and from Gary Felsenfeld gxf@VGER.NIDDK.NIH.GOV at NIH

NIH - University of Oxford (England)

National Institutes of Health Investigator:

Joseph Frank

jafrank@helix.nih.gov

University Professor:

Paul Matthews

Brain Functional Reorganization in Multiple Sclerosis

It has become well recognized that axonal and neuronal damage is associated with inflammatory lesions of multiple sclerosis. Experiments have shown that patients with multiple sclerosis have altered patterns of functional activation with motor tasks, suggesting a compensatory reorganization of motor control pathways in response to inflammatory injury. This reorganization appears to be dynamic and responsive to both changing pathology and changing functional demands. We would use functional magnetic resonance imaging in conjunction with other magnetic resonance techniques able to characterize evolution of pathology in order to better define the dynamics of the relationship between pathological structural changes and functional changes in the brain of patients with MS as new lesions develop and evolve. Coupled with this is an interest in understanding the ways in which the cortex of patients with multiple sclerosis may be involved by their disease in ways that would limit the potential for functional reorganization. Such work would be addressed primarily with use of advanced, very high-resolution magnetic resonance techniques and analysis and sophisticated image analysis approaches. This work would be suitable for students with a broad range of interests including those who wish to focus on understanding basic structural and functional neuropathology of the disease and those interested in developing improved methods for defining disease-related changes.

NIH - University of Oxford (England)

National Institutes of Health Investigator:

Charles Gerfen

gerfen@codon.nih.gov

University Professor:

Paul Bolam

Molecular Neuroanatomical Substrates of Motivated Behavior in the Forebrain

The Laboratories of Paul Bolam, at Oxford, and Charles Gerfen, at NIH, study the functional organization of the basal ganglia, with the aim to understand its involvement in a broad spectrum of mental and neurologic disorders, including Parkinson's Disease, Obsessive Compulsive Disorder, ADHD and drug abuse. The research combines molecular biology with neuroanatomical approaches to determine the organization of the neuronal networks (neuroanatomical circuits) that are involved in motivated behavior. Neuroanatomical studies in the two labs have worked out many of the principles of organization of the neuronal networks of the basal ganglia, particularly in terms of how neurochemically defined neuron types connect different parts of the basal ganglia and how the basal ganglia connect with other regions of the brain. Current work is focused on characterizing different forms of neuronal plasticity in distinct neuroanatomical pathways, which are responsible for neurologic and mental disorders. For example, we have found that in an animal model of Parkinson's Disease, a protein kinase signaling cascade that is normally under tight regulation in one of the neuronal neuroanatomical pathways within the basal ganglia becomes unregulated in the Parkinson's animal, leading to very aberrant forms of neuronal function. This aberrant response also appears in other animal models of mental and neurologic disease. Studies to be conducted under the joint Oxford/NIH program will include characterization of the neuroanatomical substrates in the basal ganglia that utilize distinct protein kinase signaling mechanisms that regulate gene expression, and the use of large scale gene expression microarrays (greater than 30,000 genes) to identify the sets of genes responsible for distinct forms of neuronal plasticity in different populations of neurons in the basal ganglia. These studies will lead to a better understanding of how the basal ganglia work and what goes wrong in disease.

NIH - University of Oxford (England)

National Institutes of Health Investigator:

Gordon Hager

hagerg@exchange.nih.gov

University Professor:

Peter Cook

Characterization of Sub-Nuclear Transcription Centers

The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor that heterodimerizes with the AhR nuclear translocator (ARNT/HIF-1ß) to form an AhR/ARNT transcription factor complex. This complex binds to specific DNA sites in the regulatory domains of numerous target genes and mediates the biological effects of exogenous ligands. Using live?cell confocal and high?resolution deconvolution microscopy, we have investigated the subcellular distribution of the AhR/ARNT complex in response to ligand stimulation [Elbi, et al. (2002) Mol. Biol. Cell (in press)]. Unliganded AhR shows a predominantly cytoplasmic diffuse distribution in mouse hepatoma cells. Upon addition of ligand, AhR rapidly translocates to the nucleus and accumulates in multiple bright foci. Dual- and triple-immunolabeling experiments, combined with labeling of nascent RNA, showed that the foci are transcription sites, indicating that upon ligand stimulation, AhR is recruited to active transcription centers. The interaction of AhR with ARNT was both necessary and sufficient for the recruitment of AhR to transcription sites. These results indicate that AhR/ARNT complexes are recruited to specific subnuclear compartments in a ligand?dependent fashion and that these foci represent the sites of AhR target genes.

A remarkable finding of our work is that a major fraction of all transcription sites are associated with the AhR/ARNT accumulation. Since it is highly unlikely that such a large faction of cellular genes are activated by the AhR/ARNT complex, these findings strongly suggest that more than one genetic locus is associated with each site of transcription. These results, in turn, are not easily resolved with a simple model where each active gene occupies a unique nuclear locus, and factors are recruited directly to that locus. The findings are more consistent with the concept of "transcription factories" advanced by Cook [Science 284:1790 (1999)]. In a direct test of these models, we will carry out FISH analysis with probes to genes that are induced by the AhR receptor, and a series of control genes that are not subject to AhR regulation. We will then compare the location of these genes with the previously characterized transcription centers. With this approach it should be possible to discriminate between the two models regarding the organization of sub?nuclear transcription sites.

NIH - University of Oxford (England)

National Institutes of Health Investigator:

John Hardy

hardyj@mail.nih.gov

University Professor:

Kay Davies

kay.davies@anat.ox.ac.uk

The Molecular Analysis of Neurological Disorders

It is now well established that the study of rare single gene disorders can often lead to important insights into the pathogenesis of more common human disorders (eg Alzheimer's disease and rare mutations in the b amyloid gene; Parkinson's disease and mutations in a synuclein). In addition, the study of mouse mutations generated through mutagenesis screens is providing new insights into genes involved in several neurological disorders. The groups at Oxford and NIH are exploiting these approaches to study neurodegenerative disease (eg Parkinson's disease, motor neuron disease) and mouse models of ataxia and behavioural traits. These projects are summarised briefly below. A student may work on any aspects of these projects which should provide a broad training in the molecular analysis of neurological disorders including siRNA, microrarrays and proteomics. The three investigators have worked together in the past so that good contact between the groups already exists facilitating a D.Phil programme which takes advantage of the strengths of both institutions.

Mouse models of human ataxia (Davies group)
We have identified mouse mutants from the ENU mouse mutagenesis programme at the MRC Mammalian Genetics Unit at Harwell which show movement disorders. We are isolating the genes responsible for these disorders and studying their function in the brain. One mutant, the robotic mouse, is caused by a dominant mutation in the AF4 gene which results in the specific loss of Purkinje cells in the cerebellum. This gene is highly homologous to the FMR2 gene which is involved in memory loss in man. We have knock-out mice for each of these genes. Both genes are thought to be transcription factors although their downstream target are unknown. These genes may functionally compensate for each other so the crossing of the two null mutants may give a clearer indication of their function. We will use laser-capture microdissection and microarray techniques to identify the genes whose regulation is altered in the dominant mutants.
Genes which are found to be downstream from AF4 and FMR2 will be investigated using in vitro techniques in tissue culture or by creating new animal models. These studies would be carried out in the Hardy laboratory where core facilities for mouse trangenics and knock-outs are available.
If genes have orthologues on the fly or the worm then their function will studies using siRNA techniques in these organisms through collaboration with groups in the MRC Functional Genetics Unit. Through the Oxford Neurogenetics Clinic (Dr Talbot) and human brain collection of ataxia and other neurodegenerative disorders, there will be opportunities to explore the potential role of relevant genes in human ataxias.

Identification of genes involved in dominant spinal muscular atrophy (Talbot group)
We have collected several large families suffering from novel forms of autosomal dominant spinal muscular atrophy, a group of pure lower motor neuron diseases. We are currently undertaking genome screening and positional cloning to map the mutations responsible for these disorders. The research programme will very soon move on to the sequencing of candidate genes and the study of their function in motor neuron survival. In order to facilitate the functional analysis of candidate genes we are developing rodent motor neuron-muscle co-culture systems which can be applied to mouse models of motor neuron diseases. We will use transgenic mouse models of human autosomal dominant SMA in this co-culture system to explore the transcriptional changes resulting from motor neuron degeneration. The ultimate aim is to identify new pathways relevant to motor neuron survival as potential therapeutic
targets for diseases such as SMA and Amyotrophic Lateral Sclerosis(ALS).

Molecular analysis of Parkinons's disease, ALS and Ataxia (Hardy group)
We have identified many families with Parkinson's disease and some with ALS/FTD, which do not show linkage to known loci, and we are currently grouping these for genetic linkage analysis (only one PD family is large enough, of itself to identify linkage). In addition, we are collecting families with novel ataxia and ALS syndromes and will pool these with those collected in Oxford.
We have an active call biology programme aimed at looking at the interactions between genes involved in the aetiology of Parkinson's disease and, as new genes are identified by us ands by others, our aim will be to try and fit their gene products into pathogenic pathways. In our core facility we will also make transgenic animal models based on the defects that are found. We will collaborate with the Davies' group in the analysis of gene expression in these mice models using the laser capture techniques followed by microarray/analysis and will use the same approaches as the Davies' group to identify downstream

targets of mutant genes. In this regard, the philosophical unity of the three labs in their approach to the understanding of neurological disease (find genes, model pathogenesis in cells and animals) ensures a high level of interaction. Previous students of the Hardy lab have moved to the Davies' lab and have moved on from their into academic careers and this will be a model for the programme we propose herein.

Interested students are invited to visit our websites and also to check out recent papers from our groups on PubMed

NIH - University of Oxford (England)

National Institutes of Health Investigator:

John Hardy

hardyj@mail.nih.gov

University Professor:

Kevin Talbot

kevin.talbot@anat.ox.ac.uk

The Molecular Analysis of Neurological Disorders

It is now well established that the study of rare single gene disorders can often lead to important insights into the pathogenesis of more common human disorders (eg Alzheimer's disease and rare mutations in the b amyloid gene; Parkinson's disease and mutations in a synuclein). In addition, the study of mouse mutations generated through mutagenesis screens is providing new insights into genes involved in several neurological disorders. The groups at Oxford and NIH are exploiting these approaches to study neurodegenerative disease (eg Parkinson's disease, motor neuron disease) and mouse models of ataxia and behavioural traits. These projects are summarised briefly below. A student may work on any aspects of these projects which should provide a broad training in the molecular analysis of neurological disorders including siRNA, microrarrays and proteomics. The three investigators have worked together in the past so that good contact between the groups already exists facilitating a D.Phil programme which takes advantage of the strengths of both institutions.

Mouse models of human ataxia (Davies group)
We have identified mouse mutants from the ENU mouse mutagenesis programme at the MRC Mammalian Genetics Unit at Harwell which show movement disorders. We are isolating the genes responsible for these disorders and studying their function in the brain. One mutant, the robotic mouse, is caused by a dominant mutation in the AF4 gene which results in the specific loss of Purkinje cells in the cerebellum. This gene is highly homologous to the FMR2 gene which is involved in memory loss in man. We have knock-out mice for each of these genes. Both genes are thought to be transcription factors although their downstream target are unknown. These genes may functionally compensate for each other so the crossing of the two null mutants may give a clearer indication of their function. We will use laser-capture microdissection and microarray techniques to identify the genes whose regulation is altered in the dominant mutants.
Genes which are found to be downstream from AF4 and FMR2 will be investigated using in vitro techniques in tissue culture or by creating new animal models. These studies would be carried out in the Hardy laboratory where core facilities for mouse trangenics and knock-outs are available.
If genes have orthologues on the fly or the worm then their function will studies using siRNA techniques in these organisms through collaboration with groups in the MRC Functional Genetics Unit. Through the Oxford Neurogenetics Clinic (Dr Talbot) and human brain collection of ataxia and other neurodegenerative disorders, there will be opportunities to explore the potential role of relevant genes in human ataxias.

Identification of genes involved in dominant spinal muscular atrophy (Talbot group)
We have collected several large families suffering from novel forms of autosomal dominant spinal muscular atrophy, a group of pure lower motor neuron diseases. We are currently undertaking genome screening and positional cloning to map the mutations responsible for these disorders. The research programme will very soon move on to the sequencing of candidate genes and the study of their function in motor neuron survival. In order to facilitate the functional analysis of candidate genes we are developing rodent motor neuron-muscle co-culture systems which can be applied to mouse models of motor neuron diseases. We will use transgenic mouse models of human autosomal dominant SMA in this co-culture system to explore the transcriptional changes resulting from motor neuron degeneration. The ultimate aim is to identify new pathways relevant to motor neuron survival as potential therapeutic
targets for diseases such as SMA and Amyotrophic Lateral Sclerosis(ALS).

Molecular analysis of Parkinons's disease, ALS and Ataxia (Hardy group)
We have identified many families with Parkinson's disease and some with ALS/FTD, which do not show linkage to known loci, and we are currently grouping these for genetic linkage analysis (only one PD family is large enough, of itself to identify linkage). In addition, we are collecting families with novel ataxia and ALS syndromes and will pool these with those collected in Oxford.
We have an active call biology programme aimed at looking at the interactions between genes involved in the aetiology of Parkinson's disease and, as new genes are identified by us ands by others, our aim will be to try and fit their gene products into pathogenic pathways. In our core facility we will also make transgenic animal models based on the defects that are found. We will collaborate with the Davies' group in the analysis of gene expression in these mice models using the laser capture techniques followed by microarray/analysis and will use the same approaches as the Davies' group to identify downstream

targets of mutant genes. In this regard, the philosophical unity of the three labs in their approach to the understanding of neurological disease (find genes, model pathogenesis in cells and animals) ensures a high level of interaction. Previous students of the Hardy lab have moved to the Davies' lab and have moved on from their into academic careers and this will be a model for the programme we propose herein.

Interested students are invited to visit our websites and also to check out recent papers from our groups on PubMed

NIH - University of Oxford (England)

National Institutes of Health Investigator:

David Harlan

davidmh@intra.niddk.nih.gov

University Professor:

Kathryn Wood

T1DM Immunology and Immunotherapy Research

Type 1 diabetes mellitus (T1DM) is a devastating illness affecting millions of people worldwide, including an estimated 1 million Americans, and 100,000 citizens in the United Kingdom. T1DM occurs as a result of the autoimmune destruction of the insulin producing beta cells in the pancreas (islets). While it is well established that individuals with T1DM can, by keeping their blood sugars normal, prevent the blindness, kidney failure, nerve damage, and premature mortality that otherwise accompanies the disease, it is also recognized that maintaining normal blood sugars by delivery of exogenous insulin is extraordinarily difficult. Replacement of the lost beta cell function through islet cell transplantation is an alternative therapeutic approach that has recently become a realistic clinical option for those already afflicted with T1DM. However, islet transplantation is currently restricted by an inadequate understanding of the ways in which the immune system responds to the transplanted islet cells, and by the too limited source of islets available for transplant.

The goal of this collaborative project is to identify novel molecular targets that can be used to develop new therapeutic strategies for the prevention of both rejection and the autoimmune destruction of islet cells after transplantion into diabetic recipients. Cellular and molecular approaches will be used to analyze the sequential activation of the immune system as it responds to donor alloantigens and islet autoantigens after transplantation to elucidate the mechanisms responsible for islet destruction. The project will take advantage of experimental models and clinical material available at the NIH and in Oxford. Integration of these data will facilitate the development of novel therapeutic agents with the potential for inhibiting immune mediated islet destruction. Participants in the Oxford - NIH Program for T1DM immunology and immunotherapy research will develop expertise in immunology, the genetics of T1DM susceptibility, and will have considerable exposure to beta cell biology.

NIH - University of Oxford (England)

National Institutes of Health Investigator:

James Hurley

james.hurley@nih.gov

University Professor:

Chris Ponting

chris.ponting@anat.ox.ac.uk

Bioinformatics and Structural Genomics of Signaling Domains

In the wake of the human genome sequence, structural genomics and bioinformatics are a powerful combination in the generation of experimentally-testable and provocative hypotheses (1). We wish to forge a link between large-scale identification of novel domains by the Ponting group (Oxford), and structure and function determination by the Hurley group (NIH). As an example of a possible project, the Ponting group previously identified a novel lipid signaling and transport domain, which they named START (2). The Hurley group went on to determine the structure of this domain and develop a model for its biochemical mechanism (3). This project seeks an ambitious individual who is keen to bridge the divide between theory and experiment, and who wishes to be trained in the latest technologies.

1. Hurley, J. H., Anderson, D. E., Beach, B., Canagarajah, B., Ho, Y., S. J., Jones, E., Miller, G., Misra, S., Pearson, M., Saidi, L., Suer, S., Trievel, R., & Tsujishita, Y. Structural Genomics and Signaling Domains. Trends Biochem. Sci. 27, 48-53 (2002).

2. Ponting, C. P. & Aravind, L. START: a lipid-binding domain in StAR, HD-ZIP and signalling proteins. Trends Biochem. Sci. 24, 130-132 (1999).

3. Tsujishita, Y. & Hurley, J. H. Structure and Lipid Transport Mechanism of a StAR-related Domain. Nature Struct. Biol. 7 , 408-414 (2000).

NIH - University of Oxford (England)

National Institutes of Health Investigator:

Sam Hwang

hwangs@mail.nih.gov

University Professor:

David Jackson

djackson@enterprise.molbiol.ox.ac.uk

The Cell and Molecular Biology of the Lymphatic System

The lymphatic system is an extensive network of capillaries and collecting vessels that permeates the body and plays a vital role in leukocyte trafficking. During immune surveillance, lymph vessels carry antigen presenting cells to lymph nodes to prime the immune response, and in cancers such as that of the breast they act as a major route for early tumour metastasis. In both these processes it is evident that cell migration is directed by the release of chemokines some released from the endothelial cells of lymphatic vessels themselves. For example the chemokine SLC produced in lymph node attracts leukocytes and tumour cells bearing the CCR7 receptor. We are interested in the mechanisms by which migrating leukocytes and tumour cells interact with lymphatic endothelial cells and whether such interactions regulate their entry and subsequent migration within the lymph vessels.

Among the candidate adhesion molecules that could mediate leukocyte-lymph vessel interactions are LYVE-1, which is expressed on lymphatic endothelial cells, and CD44 which is expressed on leukocytes. Both these proteins are receptors for hyaluronan, an extracellular matrix Mucopolysaccharide that is metabolized within the lymphatic system and that acts as a substrate for cell migration in many tissues. Also at issue is whether mucopolysaccharides themselves bind and present chemokines to migrating cells.

The project would involve analyzing the adhesive and migratory interactions between leukocytes and cultured lymphatic endothelial cells from different sources, assessing the involvement of LYVE-1/hyaluronan, and the possible regulation of these processes by chemokines.

NIH - University of Oxford (England)

National Institutes of Health Investigator:

Robert Innis

Robert.innis@nih.gov

University Professor:

Neuropsychiatric Imaging with Positron Emission Tomography

The Molecular Imaging Branch (MIB) was established by NIMH in 2000 and uses a variety of neuroimaging techniques to explore molecular and chemical mechanisms associated with neural function in health and disease. The overall goal of the Branch is to further elucidate pathophysiological mechanisms associated with neuropsychiatric disorders, with the expectation that such knowledge will ultimately decrease the burden of these illnesses by suggesting, for example, improved medication therapies.

The primary methodologies used by investigators in this Branch are PET (positron emission tomography) and NMR (nuclear magnetic resonance). Dr. Innis' area of research involves primarily PET and includes the development, evaluation, and use of new in vivo ligands to measure many different molecular targets in brain. PET ligands that are currently being used or under development include ones for the dopamine transporter, serotonin transporter, norepinephrine transporter, substance P (NK-1) receptor, dopamine D1 and D2 receptors, nicotine receptor, and amyloid (as a biomarker for Alzheimer's disease). New radiotracers are synthesized and then rigorously evaluated in animals (rodents and primates) to assess their utility to localize and quantify the functional status of their targets. Promising candidate radiotracers are extended to human subjects, first in healthy subjects and then in relevant patient populations.

The PET facilities for this Branch are extensive and include a mouse PET camera, three "standard" GE PET devices, and high-resolution head-dedicated device currently being manufactured. Studies are on going in rodents, monkeys, and humans. Several patient populations are or will soon be studied, including depression, anxiety disorders, schizophrenia, obsessive compulsive disorder, Tourette's syndrome, Parkinson's disease, and Alzheimer's disease.

NIH - University of Oxford (England)

National Institutes of Health Investigator:

Philipp Kaldis

kaldis@ncifcrf.gov

University Professor:

Jane Endicott

Jane@biop.ox.ac.uk

The structure and functions of cyclin-dependent kinases

Cyclin-dependent kinases (Cdks) are thought to promote the transitions between the different phases of the cell cycle. A number of the structural principles underlying cyclin-dependent kinase regulation have been elucidated following the determination of a series of Cdk and cyclin structures using X-ray crystallography and NMR methods. More distant members of the family that do not require cyclin association for activity, however, have so far resisted structure determination. S. cerevisiae Cdk-activating kinase (Cak1p) that phosphorylates the Cdk activation loop is an important example of this class of proteins. Intriguingly it is missing certain sequence elements usually found in the wider protein kinase family, suggesting that distinct mechanisms of action and regulation may be elucidated by structural and functional studies. This project aims to (i) carry out a structure-function study of Cak1p employing biochemical and biophysical methods (ii) determine the structure of Cak1p by X-ray crystallography (iii) determine the binding interface between Cdks and Cak1p by using full length proteins as well as peptides and (iv) integrate these studies to probe the in vivo function and regulation of Cak1p.

We envisage that the structural analysis will be performed in Oxford and the biochemistry at the National Cancer Institute, Frederick.

NIH - University of Oxford (England)

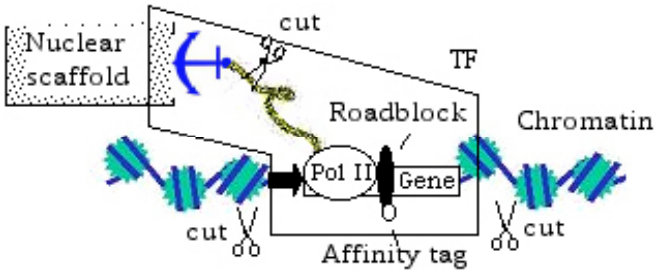
National Institutes of Health Investigator:

Mikhail Kashlev

mkashlev@mail.ncifcrf.gov

University Professor:

Peter Cook



Isolation and Biochemical Characterization of 'Transcription Factories from the Yeast S.cerevisiae

The modern concept of eukaryotic transcription suggests that RNA synthesis in nucleus takes place in the stationary "transcription factories " ((TFs)containing a pool of immobilized RNA polymerase II (Pol II)molecules attached to the solid nuclear elements. Transcription by these polymerases may involve temporary recruitment of the genes to these sites.In these factories, transcription is believed to occur by threading the template through the immobilized enzyme.The protein composition of the TFs is extremely complex and highly dynamic.It has been shown that the TFs may contain RNA polymertase,transcription elongation factors, mRNA modification,processing/splicing machinery,and components of nuclear translation apparatus.

Our project involves biochemical characterization of the TF, assembled at unique yeast (S.cerevisiae)gene in vivo .For the TF isolation,we developed a novel technology for "halting " and stabilizing the TF in vivo by specially designed transcription roadblocks,followed by the TF purification using an affinity tag, introduced to one of the TF components.Upon further development,the project will involve comparison of the TFs assembled on different yeast genes,or the factory assembled on the same gene,but in different chromosome locations.

The main elements in the project are outlined in the attached Schemes 1 and 2.The detailed description of the project is available upon request.

Figure Legend
In order to accumulate and stabilize the TF and to preserve its protein content, the factory will be switched to a "halted" mode by introduction of a protein roadblock (lac repressor bound to its operator sequence: shown by black oval) at the end of the yeast Ty1 transposable element. Ty1 will be expressed from the inducible GAL1 promoter (shown by black arrow) in the yeast S. cerevisiae cells. The roadblock will stop the leading Pol II molecule and will induce traffic "jam" within the TF, causing accumulation of the polymerase elongation complexes and associated factors within the gene.

The TF-Ty purification scheme will include two principal innovations. Affinity purification of the Ty1 DNA will be achieved using biotin tag in the lac repressor (LacI). The biotin group will be introduced to LacI in vivo by the activity of biotin-ligase BirA, which will be co-expressed in the same cell. This tag will allow separation of the TF-Ty factory from all other factories in the cell using absorption to streptavidin agarose beads. The dissociation of the repressor-operator complex with IPTG will provide a highly selective way for elution of the TF from the beads.

We anticipate purification problems caused by the tangled chromatin environment in the nucleus. Therefore, we plan to untangle the TF-Ty by cutting it out of chromatin with HO endonuclease (symbolized by scissors). For that purpose, the HO cutting sites will be introduced at both flanks of the Ty element. The plasmid-born HO nuclease will be temporarily expressed in the same cells at the later steps of the TF accumulation.

NIH - University of Oxford (England)

National Institutes of Health Investigator:

Allan Kirk

allank@intra.niddk.nih.gov

University Professor:

Kathryn Wood

Transplantation Tolerance

When patients undergo an organ transplant procedure, they are required to take immunosuppressive medications for life to prevent immune rejection of the transplanted organ. These drugs are relatively non-specific and exact a significant cost in terms of infectious, malignant and physiological side effects. Conventional wisdom has held that since the immune system causes rejection, it must be suppressed to prevent graft loss. My lab is showing otherwise.

The immune system is not an offensive system void of regulation. Rather, it is tightly regulated to provide protective immunity through measured responses to specific threats to homeostasis. As such, it must down regulate responses as well as augment them, and is equally capable of both preventing and promoting transplant rejection. My research has thus been directed toward understanding the regulatory aspects of immunity and exploiting them to achieve transplant tolerance - a state in which the immune response favors acceptance of an organ rather than rejection. Students applying to work in my laboratory should have a strong interest in immunology particularly as it relates to the study of tolerance.

The primary goals of persons working in my lab lie in the transition of promising therapies from the laboratory into proof of concept clinical trials. My group thus uses cellular and molecular in vitro assays, and both rodent and non-human primate models of transplantation to model therapies for initial clinical use. Therapies that show promise pre-clinically are investigated in humans at the Clinical Center under approved renal transplant protocols.

We are currently investigating several methods for tolerance induction. One critical regulatory pathway involved in T cell immunity involves the costimulation receptor-ligand pair CD40: CD154. We have been successful in targeting CD154 with monoclonal antibodies to prevent allograft rejection in non-human primates without chronic immunosuppression, and are now evaluating multiple sources of anti-CD154 pre-clinically. Of particular interest is the expression of CD154 on activated platelets and the implications this has for immune activation caused by surgical trauma. We are particularly focused on platelet-monocyte interactions with the hypothesis that trauma induced platelet activation contributes to initial antigen presenting cell activation and maturation. Other costimulatory molecules being investigated include the B7 molecules CD80 and CD86.

We have initiated two clinical trials based on the concept that transient immune depletion prevents trauma induced alloimmune activation, and skews the alloimmune response towards tolerance rather than rejection. Using the monoclonal antibody Campath-1H, or alternatively the polyclonal antibody preparation Thymoglobulin to achieve transient T cell depletion prior to allograft reperfusion, we have been able to substantially reduce the need for postoperative immunosuppression in humans. This is presumably due to the avoidance of antigen presentation to T cells at the peak of immune activation (the surgical procedure itself). We are now modelling several variations of this approach in non-human primates to understand how a reconstituting immune system engages a transplanted organ. Again, monocyte activation plays a key role in this response and we are evaluating human allograft derived monocyte populations to gain clues into their regulation at the time of a traumatic insult. CD40 ligation clearly plays a role in this approach as well, though responses to reperfusion associated cytokines and responses to graft derived cellular debris or apoptosis appear to be important immune modulates that are receiving critical attention.

Selected Publications:

Kirk AD, Harlan DM, Armstrong NN, et al CTLA4-Ig and anti-CD40 ligand prevent renal allograft rejection in primates. Proceeding of the National Academy of Sciences USA 94:8789-8794, 1997.

Kirk AD, Burkly LC, Batty DS, et al. Humanized anti-CD154 monoclonal antibody treatment prevents acute renal allograft rejection in non-human primates Nature Medicine 5:686-693, 1999.

Kirk AD, Tadaki DK, Celniker A, et al. Induction Therapy with Monoclonal Antibodies Specific for CD80 and CD86 Delays the Onset of Acute Renal Allograft Rejection in Non-human Primates Transplantation 72:377-84, 2001.

Elster EA, Xu H, Tadaki DK, Montgomery S, et al Treatment with the Humanized CD154-specific Monoclonal Antibody hu5C8 Prevents Acute Rejection of Primary Skin Allografts in Non-Human Primates Transplantation 72:1473-1478, 2001.

Kirk AD Transplant Tolerance: a look at the non-human primate literature in the view of modern tolerance theories Critical Reviews in Immunology 19:349-88, 1999.

NIH - University of Oxford (England)

National Institutes of Health Investigator:

Alan Koretsky

koretskya@ninds.nih.gov

University Professor:

Paul Matthews

Functional and structural parcellation of the human cortex

Classical histological studies have established that the neocortex of the mammalian brain highly heterogeneous. Structural differences between regions have been described based on cellular organisation(cytoarchitecture) and the amount and arrangement of myelinated fibres(myeloarchitecture). These structural differences are likely to reflect functional differences. Understanding and being able to measure non-invasively such "fine" structure on individuals is an important step towards developing improved understanding of the way complex brain networks for brain computation develop and are organized. We have begun to use a variety of magnetic resonance-based strategies in order to develop ways of providing fine structural analysis of both the human and rodent neocortex in vivo. Approaches using high field magnetic resonance imaging with development of improved methods for defining contrast based on differences in structural characteristics will be developed as part of the project. Novel strategies for assessing connectivities between different brain areas based on diffusion tensor imaging and Manganese Enhanced MRI could provide additional information on neocortical structure based on differences in connectivities with other brain regions. Functional imaging could further contribute to distinguishing meaningful regional discrimination. A focus on analysis of individual brains would allow an appreciation of individual variation across populations, potentially establishing a basis for use of imaging markers for phenotyping in genetic studies of neurodevelopment. A graduate student from the Oxford/NIH program would be able to work on aspects of this project at both FMRIB (Paul Matthews, Director) in Oxford and the Laboratory of Functional and Molecular Imaging (www.lfmi.ninds.nih.gov Alan Koretsky, Director) in the National Institute of Neurological Disorders and Stroke at NIH. The LFMI has unique MRI at very high field strengths for animals and humans as well as interests in detecting cortical heterogeniety with hi resolution anatomical, functional and molecular imaging.

NIH - University of Oxford (England)

National Institutes of Health Investigator:

Alan Koretsky

koretskya@ninds.nih.gov

University Professor:

Kieran Clarke

Kieran.Clarke@bioch.ox.ac.uk

Energetics, Ions, and Transcription Factors in the Failing Heart

Insulin resistance and abnormal cardiac energetics are found in heart failure patients and, we hypothesize, reflect the same underlying pathogenic mechanism, energy substrate depletion. In this project, the effect of substrates and insulin on energetics, contractile function and efficiency will be studied in the chronically failing mouse heart. At the University of Oxford, cardiac function, intracellular pH, and energetics will be measured under normal and acute (ischaemia/reperfusion) or chronic (exercise) stress conditions, using magnetic resonance imaging and spectroscopy.1 Changes in peroxisome proliferator-activated receptors (PPARs), members of the nuclear hormone receptor family, will be determined in the failing heart. PPARs regulate gene expression by heterodimerizing with retinoid X receptors (RXR), then binding to peroxisome proliferator response elements in the promoter region of target genes controlling lipid and glucose metabolism.2 Substrate oxidation, O2 consumption, mitochondrial respiration and uncoupling will also be measured.3 At the NINDS, manganese-enhanced MRI will be used to determine the rate of calcium influx into the failing mouse heart and mitochondrial protein changes will be determined using difference gel electrophoresis.4,5 We propose that the transition to failure involves changes in fatty acid oxidation, nuclear transcription factors, mitochondrial uncoupling, ATP synthesis and glycolysis, with energy substrate depletion the outcome.

References

1. King LM, Sidell RJ, Wilding JR, Radda GK, Clarke K. Free fatty acids, but not ketone bodies, protect diabetic rat hearts during low-flow ischemia. Am J Physiol 280:H1173-H1181, 2001.
2. Sidell RJ, Cole MA, Draper NJ, Desrois M, Buckingham R, Clarke K. Thiozolidinedione treatment normalizes insulin resistance and ischemic injury in the Zucker fatty rat heart. Diabetes In press. 2001.
3. Boehm EA, Jones BE, Radda GK, Veech RL, Clarke K. Increased uncoupling proteins and decreased efficiency in the palmitate-perfused hyperthyroid rat heart. Am J Physiol 280:H977-H983, 2001.
4. Hu TC-C, Pautler RG, MacGowan GA, Koretsky AP. Manganese-enhanced MRI of mouse heart during changes in inotropy. Magn Reson Med 46:884-890, 2001.
5. Kernec F, Unlu M, Labeikovsky W, Minden JS, Koretsky AP. Changes in the mitochondrial proteome from mouse hearts deficient in creatine kinase. Physiol Genomics 6:117-128, 2001.

NIH - University of Oxford (England)

National Institutes of Health Investigator:

Richard Koup

rkoup@mail.nih.gov

University Professor:

Sarah Rowland-Jones

rowland-jones@ndm.ox.ac.uk

Phenotypic Characterisation of HIV Specific CTLs

Virus specific cytotoxic T-lymphocytes (CTL) are widely believed to play an important role in the control of HIV replication. Some of the evidence for this includes an association between vaccine-induced CTL and protection from SIV challenge in monkey models and the observation that a significant proportion of people with HIV exposure who do not develop persistent infection generate an HIV-specific CTL response in the absence of circulating antibodies to the virus. However, the majority of HIV-infected people develop disease and die despite a strong virus-specific CTL response. This paradox cannot be explained by a quantitative difference in virus-specific CTL numbers but may be a consequence of qualitative differences in T-cell phenotype, such as cytokine or chemokine production, lytic ability, susceptibility to apoptosis and so on. The support of the CTL response by HIV-specific helper T-cells, characteristically deficient in HIV infection, may also play a key role.

In this project we propose to generate a panel of HIV-specific CD4+ and
CD8+ T-cells from HIV-infected donors, HIV-exposed but persistently seronegative (HEPS) donors and recipients of a prime-boost vaccine approach designed to elicit a cellular immune response, and then to define the phenotype and functional characteristics of these cells. The project will be carried out in a collaboration between the laboratories of Professors Rick Koup in the Vaccine Research Centre, NIH and Sarah Rowland-Jones in the Human Immunology Unit in Oxford.

NIH - University of Oxford (England)

National Institutes of Health Investigator:

Michael Lenardo

lenardo@nih.gov

University Professor:

Gavin Screaton

screaton@molbiol.ox.ac.uk

Molecular regulation of cell death by the Tumor Necrosis Factor (TNF) Receptor gene superfamily

Apoptosis is a fundamental process involved in the development and function of many systems in multicellular organisms. In the immune system apoptosis is used as an effector mechanism by cytotoxic cells and is also of central importance to lymphocyte homeostasis in the thymus and periphery. Much has been recently learnt about the molecular pathways signaling cell death. One such pathway is initiated by the ligation of cell surface death receptors, members of the TNF receptor superfamily by their ligands which belong to the TNF superfamily. Although the signaling pathway activated from these receptors has been extensively studied little is known about the macromolecular structures formed at the cell surface to initiate the signal. The recent demonstration that receptors can self associate before contact with ligand provides an interesting lead and may underlie the formation of supra-molecular complexes necessary for signaling. Further understanding of the cell surface interactions necessary for signaling may allow the generation of pharmacologically active small molecules to inhibit cell death in pathological situations.

In this project it is proposed to use a variety of biophysical, structural and cellular techniques to study interactions between death receptors and their ligands at the cell surface. One of the goals will be to define the "apoptotic synapse" formed when two cells expressing death receptors or their ligands come into contact.

NIH - University of Oxford (England)

National Institutes of Health Investigator:

Michael Lenardo

lenardo@nih.gov

University Professor:

Gavin Screaton

screaton@molbiol.ox.ac.uk

Molecular mechanisms of how HIV kills CD4+ T lymphocytes in the pathogenesis of AIDS

A principal event underlying the fatal immunodeficiency caused by the Human Immunodeficiency Virus (HIV) is its cytopathic effect on CD4+ T lymphocytes. It is believed that the inability of the immune system to eradicate HIV stems in large part from the failure of adequate helper T cell responses due to the loss of HIV-specific CD4+ T cells during both acute and chronic infection. This could also be an impediment to effective therapeutic vaccination. The recovery of helper T cell responses and the attendant immunological recovery that follows viral protease inhibition indicates that therapies directed at preventing viral cytopathicity could be valuable for enhancing the natural immune amelioration or elimination of HIV infection. Although many mechanisms have been proposed, the precise events in HIV cytopathicity are poorly characterized in molecular terms. The goal of this project is to identify the molecular pathway by which HIV causes the death of T lymphocytes. A combined biochemical and molecular approach will be used to first identify the viral functions that cause death and then identify their cellular targets. The elucidation of molecular targets involved in T cell death should promote the future development of novel pharmaceutical compounds that can interfere with the ability of the virus to cause cell death and thereby complement the action of presently existing anti-viral compounds.

NIH - University of Oxford (England)

National Institutes of Health Investigator:

Michael Lenardo

lenardo@nih.gov

University Professor:

John Bell

john.bell@ndm.ox.ac.uk

The Identification and Characterisation of Pathways that Control Immune Responses to Antigen

Introduction
The success of the human genome mapping project and other recent advances in genetics have placed us in an unrivalled position to study the function of genes and the inherited basis of human disease; but, given the number of genes, there needs to be an efficient way to focus on those genes and pathways that are essential for key processes of medical relevance.

To address this problem, Oxford University and the Australian National University in Canberra have been funded by the Wellcome Trust to generate loss-of-function mutations in large numbers of genes in parallel using ENU mutagenesis, and screen the mice with these mutations for the sub-set of genes that are essential for lymphocyte responses to antigen. Over the past 30 years a limited repertoire of highly informative spontaneous mouse mutants, such as xid and fas/lpr have been at the cornerstone of immunology and entry points for understanding new biochemical pathways and function. Mike Lenardo's group at the NIH has been at the forefront of demonstrating how such information can be applied to understanding human disease susceptibility.

Our core programme started in April 2001 and we are now identifying new mutant strains that will be available for detailed analysis in the Michaelmas term of 2002. The strains can be characterised using a range of downstream assays and experimental systems, including DNA microarrays, and the net result of this research will be to connect genes and function within molecular and cellular pathways. Many of the mutated proteins will lie in the biochemical pathways regulating important immune functions, and the characterization of these pathways may lead to new targets for immunotherapy. Amongst the several strains in our pilot study have been animals with defective negative regulation of immune responses, including mice with lymphadenopathy which is not due to lymphoma. In conjunction with Mike Lenardo's group in the NIH we aim to study the functional and genetic defects in new strains of mice with exaggerated responses to exogenous or self-antigens.

Plan of Research
1. Selection of Mutants. Mutant C57BL/6 lines with exaggerated responses to antigen will have been identified using a screening strategy carried out in Australia that is based on flow cytometry, oxazolone hypersensitivity reactions, humoral immunity, T-dependent (Th1 and Th2) immunizations and an in vitro assay of CD4-mediated T cell proliferation to antigen using antigen-specific transgenic T cell clones ex vivo. We will also identify mice with high spontaneous anti-nuclear antibody titres. From our experience, we would like the student to undertake the characterisation of at least 3 unique mutants strains. As part of the collaboration we would expect the student to spend some time in Chris Goodnow's laboratory in Australia.
2. Gene Mapping and Sequencing. The student will map and sequence the new mutations. The mapping will be done by 100 animal backcrosses, and the sequencing will be undertaken from cDNAs within regions of linkage. We use radiation chimeras to identify the immune cells carrying the mutations in cis. These aspects of the programme have also been extensively piloted and aill not be unduly complex..
3. Functional Analysis. The student will next analyse functional effects of the mutations in the laboratory using a range of assays and mouse models of tolerance. This is clearly the most challenging and exciting part of the programme and will give rise to the opportunity for collaborations with other groups in Oxford, the NIH and elsewhere. The Nuffield Department of Medicine and Oxford University have wide resources and expertise in all many of immunology and genetics.
4. Expression Arrays. The student will also characterise the affected cells ex vivo using oligonucleotide expression arrays, which have been developed to support the ENU programme.

Training Aspects
This project is suitable for someone who wants to bridge the gap between genetics and functional biology. It will give experience in techniques using whole animal models, cellular biology, conventional genetics, molecular biology and some bioinformatics. There will be extensive support and training from other parts of the ENU programme, including the core facilties (arrays, mapping et cetera). The Department of Medicine, the Weatherall Institute of Molecular Medicine and the Wellcome Trust Centre for Human Genetics host regular seminar series, as well as lecture courses for students. From November 2003, the research will take place in a new Centre for Cellular and Molecular Physiology in Oxford, which is being established to foster collaborations between groups using various genetic, biochemical and physiological approaches to

understand complex systems.

NIH - University of Oxford (England)

National Institutes of Health Investigator:

Michael Lenardo

lenardo@nih.gov

University Professor:

Katja Simon

katja.simon@clinical-medicine.oxford.ac.uk

The Role of Autophagy in the Developing and Mature Immune System

Autophagy is a complex programme responsible for lysosomal degradation of cytoplasmic proteins and organelles; it is often activated in response to nutrient deprivation, leading to recycling of cytoplasmic contents to provide metabolic precursors, and can be essential for survival. Autophagy is also involved in programmed cell death. When apoptosis is inhibited by caspase inhibitors, cells die by autophagy [1]. Autophagy is characterised by double membrane extra-nuclear vesicles called autophagosomes engulfing bulk cytoplasm and organelles; in yeast, several evolutionarily conserved atg genes encode essential mediators [2]. Beclin-1 is a mammalian homologue of Atg6, it is thought to mediate the localisation of autophagy proteins to pre-autophagosomal structures. The disruption of both alleles of beclin-1 in mice is embryonically lethal. However, beclin+/- mice show an increased frequency of spontaneous malignancies [3, 4] and reduced autophagy in vivo. Notably, from an immunological point of view, the heterozygous mice have an increased frequency of extranodal, splenic and nodal cellularity. Such increased cellularity is also seen in the thymic medulla, associated with germinal centres. Interestingly, thymic germinal centers are typical in autoimmune diseases, such as in ageing NZB mice with autoimmune anaemia [5], in early-onset myasthenia gravis patients and in systemic lupus erythematosus (SLE) [6].

Moreover, in transgenic mice whose autophagosome marker - microtubule associated light chain 3(LC3) - was fused to GFP, LC3 was strongly and constitutively expressed in medullary thymic epithelium (MTECs) [7]. This Atg8 homologue redistributes characteristically as ring-shaped structures or dots during autophagy [8]. In the thymus, GFP-LC3 dots were observed in both the cortex and medulla; the positive cells were clearly epithelial (cytokeratin+) confirmed by immunoelectron microscopy. Deficient autophagy in MTECs might cause deficient autoantigen presentation and self-tolerance. The peripheral increased cellularity could be a consequence of this thymic defect (eg, of any resulting autoimmunity); or it could arise independently in the periphery. We are planning to analyse autoimmune signs in the periphery of beclin+/- mice. These will be analysed using a commercially available kit for detecting autoantibodies. Also, sections of testis, liver, intestine, heart, thyroid gland, salivary gland, pancreas and kidney will be stained for infiltrating lymphocytes. Finally, the susceptibility of beclin+/- mice to induced autoimmunity will be tested in an established model of experimental allergic encephalomyelitis (EAE).

Finally, our own preliminary analysis of the beclin +/- mouse revealed that increased cellularity in the spleen is possibly not due to T or B cell lymphoproliferation but to the proliferation of a cells of the myeloid lineage, perhaps macrophages or dendritic cells.

We will identify the over-proliferating cell population by fluorescent activated cell sort (FACS). Subsequently we will generate transgenic mice deficient for beclin-1 in the identified cells using the CreLox system. This will allow us to make tissue specific homozygous mice deficient for beclin-1. The role of autophagy in the developing and mature adaptive immune system has not been the subject of any study so far and opens exciting new avenues in the understanding of survival and death in immune tolerance and homeostasis.

The experience in the field of thymic selection, transgenic mouse models and apoptosis that we have gained over the past years and the strong interest in autoimmunity in the Weatherall Institute of Molecular Medicine makes this a very feasible and attractive project. The animal house has recently been refurbished and has an excellent track record for the generation of transgenic mice.

1. Yu, L., A. Alva, H. Su, P. Dutt, E. Freundt, S. Welsh, E.H. Baehrecke, and M.J. Lenardo, Regulation of an ATG7-beclin 1 program of autophagic cell death by caspase-8. Science, 2004. 304: p. 1500-1502.
2. Shintani, T. and K. D.J., Autophagy in Health and Disease: A double edged Sword. Science, 2004. 306: p. 990-994.
3. Yue, Z., S. Jin, C. Yang, A.J. Levine, and N. Heintz, Beclin 1, an autophagy gene essential for early embryonic development, is a haploinsufficient tumor suppressor. Proc Nat Acad Sci, 2003. 100: p. 15077-15082.
4. Qu , X., J. Yu, G. Bhagat, N. Furuya, H. Hibshoosh, A. Troxel, J. Rosen, E.L. Eskelinen, N. Mizushima, Y. Ohsumi, G. Cattoretti, and B. Levine, Promotion of tumorigenesis by heterozygous disruption of the beclin 1 autophagy gene. J Clin Inv, 2003. 112: p. 1809-1820.
5. East, J., M.A. de Sousa, and D.M. Parrott, Immunopathology of New Zealand Black (NZB) mice. Transplantation,

1965. 3: p. 711-729.
6. Roxanis, I., K. Micklem, and N. Willcox, Thymic myoid cells and germinal center formation in mysthenia gravis; possible roles in pathogenesis. J Neuroimmunol, 2002. 125: p. 185-197.
7. Mizushima, N., A. Yamamoto, M. Matsui, T. Yoshimori, and Y. Ohsumi, In vivo analysis of autophagy in response to nutrient starvation using transgenic mice expressing a fluorescent autophagosome marker. Mol Biol Cell, 2004. 15: p. 1101-1111.
8. Kabeya, Y., N. Mizushima, T. Ueno, A. Yamamoto, T. Kirisako, T. Noda, E. Kominami, Y. Ohsumi, and T. Yoshimori, LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosome membranes after processing. EMBO J, 2000. 19: p. 5720-5728.

NIH - University of Oxford (England)

National Institutes of Health Investigator:

David Levens

levens@helix.nih.gov

University Professor:

Alexandre Akoulitchev

alexandre.akoulitchev@path.ox.ac.uk

Interplay of Activators and Repressors with TFIIH: Regulating early transcription in health and disease

The early stages of mRNA transcription occur under the stewardship of multi-component complexes and subcomplexes. Among these factors, TFIIH supplies two helicases (XPB and XPD) and a cyclin dependent kinase (CDK7) that phosphorylates the C-terminal domain (CTD) of the largest subunit of RNA polymerase II. A transcriptional activator (FBP) and a repressor (FIR) work through TFIIH to control transcript extension. It is likely that TFIIH serves as a regulatory node receiving multiple signals and transducing this information through its enzymatic activities to gate the holdback or extension of nascent transcripts at multiple stages prior to promoter escape.

One aspect of this collaborative project would be to screen nuclear extracts for alternative factors that regulate p89 helicase activity of TFIIH. The question would be whether FBP(FIR) are the major factors with such activities or not? And if there are others, how do the TFIIH mutations giving rise to the diseases xeroderma pigmentosum, Cockayne's syndrome and trichothiodystrophy effect their activity? The role of TFIIH in the regulation of growth and proliferation will be explored. TFIIH-dependent targets will be identified. These experiments will employ a variety of biochemical, cell biological, genetic and bioinformatic approaches.

NIH - University of Oxford (England)

National Institutes of Health Investigator:

David Levens

levens@helix.nih.gov

University Professor:

Nicholas Proudfoot

nicholas.proudfoot@pathox.ac.uk

Factors regulating transcriptional transitions from initiation through termination

mRNA synthesis requires orderly progression through the various stages of transcription (pre-initiation complex formation, initiation, promoter escape, elongation and termination), as well as coordination with RNA processing events. Moreover it is reasonable to expect that the synthesis of most mRNAs is influenced by particular biological parameters conferring gene specific regulation. We will use biochemical fractionation and an immobilized template assay to study transcription regulation at several of the key transitions of mRNA synthesis. Particular focus will be placed upon the factors regulating the promoter escape to elongation and elongation to termination transitions. New factors identified by these in vitro approaches will also be analyzed using in vivo systems, by knocking out their gene homologs in either yeast or chicken DT40 cells since both such systems allow efficient gene modification by homologous recombination.

hnRNP K is a multi-functional protein coordinating several processes in mRNA biogenesis. Binding sequence specifically and with high affinity to both single-stranded DNA (occurring either in response to torsional strain or due to unwinding during transcription) and RNA, hnRNP K has properties indicating that it plays an important role in mRNA biochemistry. A variety of biochemical, cell biological, and genetic approaches will be used to assess its role in gene regulation.

NIH - University of Oxford (England)

National Institutes of Health Investigator:

David Levens

levens@helix.nih.gov

University Professor:

Alexandre Akoulitchev

alexandre.akoulitchev@path.ox.ac.uk

Regulation of General Transcription/Repair Factor TFIIH

The following project offers an extended biochemical and cytological analysis of TFIIH, a general transcription and DNA repair factor. The regulation of all four enzymatic activities present in TFIIH and its function in RNA Polymerase II transcription, general nucleotide- and base-excision repair and transcription-coupled repair are to be analysed with regard to well documented mutations in its subunit p89 (XPB). Genetic defects in this subunit can lead to three distinct hereditary syndromes - Xeroderma Pigmentosum (XP), Cockayne Syndrome (CS) and Trichothiodystrophy (TTD). Only in the case of XP are the genetic defects associated with increased (up to 2000-fold) susceptibility to skin cancer. Preliminary data clearly indicates the presence of a number of novel factors that can interact with and regulate the helicase activity of p89, which is essential for transcriptional activity of TFIIH. The goal of this study is to purify and characterize the newly identified activities that interact with and regulate TFIIH and its subunit p89. The regulation of TFIIH function(s) would be analyzed with regard to the genetic defects in p89(XPB). Analysis of these factor(s) and their role in transcription, transcription-coupled and general DNA repair will help better understand the physiology of natural defects in TFIIH and the biological mechanisms they affect with respect to skin cancer.

NIH - University of Oxford (England)

National Institutes of Health Investigator:

Eric Long

eLong@nih.gov

University Professor:

Gillian Griffith

The Immunological Synapse of Natural Killer Cells

Natural killer (NK) cells secrete cytokines and initiate cytotoxicity upon contact with certain tumor cells and virus-infected cells. In contrast to cytotoxic T cells that rely primarily on the antigen-specific receptor for target cell recognition, NK cells utilize an array of activating and inhibitory receptors to control cytokine secretion and target cell lysis. The immunological synapse formed at the NK cell - target cell interface and the importance of receptor distribution for signaling cytotoxicity are not well known. High resolution imaging of fixed and of live NK - target cell conjugates will be used to visualize dynamic interactions between NK cells and target cells. The goal of the project is not only to describe the movement and organization of receptors and their ligands at the NK - target cell interface but also to define signals that regulate these interactions and, ultimately, determine the type of NK cell response.

NIH - University of Oxford (England)

National Institutes of Health Investigator:

Chris McBain

mcbainc@mail.nih.gov

University Professor:

Ole Paulsen

ole.paulsen@physiol.ox.ac.uk

Network Oscillations, Interneurons and Synaptic Plasticity in the Hippocampus

A project to investigate the role of specific neural elements within the mammalian hippocampus and cortical formations in network oscillations and synaptic plasticity is offered. Specifically this collaborative project will utilize patch clamp electrophysiological techniques, immunohistochemistry and modeling of small circuits comprised of well defined interneuron:principal cell elements. Synaptic mechanisms and intrinsic properties of individual elements in the network will be explored. In addition, the roles of short and long term synaptic plasticity at individual excitatory and inhibitory synapses in modulating large-scale network oscillatory activity will be investigated. The use of multi-site recording techniques combined with high resolution patch clamping and computational modeling will be a central component of the proposed project.

NIH - University of Oxford (England)

National Institutes of Health Investigator:

Maximilian Muenke

mmuenke@nhgri.nih.gov

University Professor:

Andrew Wilkie

Craniofrontonasal Syndrome and the X Chromosome - A Paradoxical Inheritance Pattern

Medical textbooks teach us that X-linked diseases are normally more severe in males than females. Craniofrontonasal syndrome (CFNS) challenges these established notions: this X-linked disorder typically affects females (who have a combination of frontonasal dysplasia and craniosynostosis), whereas males have only mild features. Various explanations of this paradoxical inheritance have been proposed; this project seeks to identify the gene mutated in CFNS and hence to establish the correct biochemical mechanism. CFNS is the most common craniosynostosis syndrome for which the molecular basis is unknown and combines several distinct developmental abnormalities, providing additional incentives to identify the genetic basis.

This project will involve a wide range of human genetics techniques, leading to functional analysis once the mutated gene is identified. Both mentors have extensive experience of these approaches and have previously collaborated on this and similar projects.

Websites:
Dr Muenke: http://www.genome.gov/page.cfm?pageID=10000793
Prof. Wilkie: http://www.imm.ox.ac.uk/pages/research/clinical_genetics/one.htm

Reference:
Feldman GJ, Ward DE, Lajeunie-Renier E, Saavedra D, Robin NH, Proud V, Robb LJ, Der Kaloustian V, Carey JC, Cohen MM Jr, Cormier V, Munnich A, Zackai EH, Wilkie AOM, Price RA & Muenke M (1997). A novel phenotypic pattern in X-linked inheritance: craniofrontonasal syndrome maps to Xp22. Hum Mol Genet 6:1937-1941.

NIH - University of Oxford (England)

National Institutes of Health Investigator:

Andre Nussenzweig

andre_nussenzweig@nih.gov

University Professor:

Ian Hickson

i.hickson@icrf.icnet.uk

Molecular basis for genomic instability in cancer prone disorders

Heretidary diseases affecting the cellular response to double strand breaks include ataxia telangiectasia (ATM mutated), Nijmegen breakage syndrome (Nbs1 mutated), and Bloom' syndrome (BLM mutated). The hallmarks of these disorders include growth defects, immunodeficiency, hypogonadism, sensitivity to specific DNA damaging agents, defects in telomere metabolism, and cancer predisposition. The finding that ATM, Nbs1, and BLM reside in a large protein complex that includes the breast cancer susceptibility gene (brca1) and several mismatch repair proteins may provide an explanation for the similarities among these chromosomal instability disorders. However, little is known about how ATM, Nbs1 and BLM function in the repair of damaged DNA.

In this project, it is proposed to use yeast and mouse genetics to study the mechanisms by which BLM, ATM and Nbs1 function in non-homologous end joining and homologous recombination pathways of DNA repair. Potential roles that these proteins play in regulating sub-nuclear localization of repair/recombination proteins and signaling cell cycle checkpoints will also be investigated.

NIH - University of Oxford (England)

National Institutes of Health Investigator:

John O'Shea

osheajo@mail.nih.gov

University Professor:

Fiona Powrie

fiona.powrie@path.ox.ac.uk

Dr Fiona Powrie's Mucosal Immunology Group (Oxford) conducts research into mucosal immune regulation, T cells in inflammatory bowel disease, regulatory T cells, and the role of the bacterial flora in intestinal inflammation.

Foreign transplanted tissues are subject to rejection processes initiated by T-cells. In a range of rodent models we have been able to show that a short course of treatment with monoclonal antibodies that "blockade" the immune system (e.g. CD4 and CD8) can result in long-term acceptance of the graft and reprogramming of the immune system towards tolerance. Tolerance, so induced is "dominant" and actively mediated by CD4+ T-cells which seem able to prevent generation of proper T-cell effector function. Such T-cells localise in the tolerated tissue, and may express a number of genes at that site which may influence the extent of inflammation that is permitted. Not only do such regulatory T-cells stop immune responses to the tolerated antigen, but also to other antigens located in the same micro-environment. The pharmacological basis for dominant tolerance and linked suppression is currently unresolved.

1. Diana Zelenika, Elizabeth Adams, Sue Humm, Luis Graca, Sara Thompson, Stephen P. Cobbold, and Herman Waldmann. Regulatory T Cells Overexpress a Subset of Th2 Gene Transcripts J Immunol 2002 168: 1069-1079

2. Waldmann, H. and Cobbold, S. 2001 Regulating the immune response to transplants: a role for CD4+ regulatory cells? Review Immunity 14: 399-406

3. Zelenika,D., Adams, E., Mellor, A., Simpson, E., Chandler, P., Stockinger, B., Waldmann, H., & Cobbold, S. P. 1998 Rejection of H-Y disparate skin grafts by monospecific CD4+ T helper 1 (Th1) and T helper 2 (Th2) cells: no requirement for CD8+ T cells or B cells. J. Immunol. 161: 1868-1874.

4. Waldmann, H., & Cobbold, S. 1998. How do monoclonal antibodies induce tolerance? A role for Infectious Tolerance? Ann. Rev. immunol. 16: 619-44.

NIH - University of Oxford (England)

National Institutes of Health Investigator:

John O'Shea

osheajo@mail.nih.gov

University Professor:

Fiona Powrie

fiona.powrie@path.ox.ac.uk

Molecular and Cellular Biology of Regulatory T Cells

It is well established that cytokines regulate all aspects of lymphoid development and function. Although interleukin (IL)-2 was one of the first cytokines to be discovered, its in vivo function remains difficult to characterize. It is the prototypic T cell growth factor but deficiency of IL-2 results in autoimmune disease. Thus, IL-2 appears to have essential functions in promoting peripheral tolerance. The mechanisms that underlie this tolerance are poorly understood, however. One mechanisms thought to be involved is the regulation of activation-induced cell death, though again, IL-2 has very paradoxical functions in terms of regulation of apoptosis with both pro- and anti-apoptotic actions. Another, non-mutually exclusive mechanism is through the action of CD4+, CD25+ regulatory T cells (Treg), which apparently require IL-2 for their development and/or function. This project joins the forces of two groups with complementary expertise. The focus of Dr. O'Shea's group is the biochemistry and molecular biology of cytokine signal transduction. Dr. O'Shea's group discovered a kinase, Jak3 that is responsible for initiating signaling via the IL-2 and related receptors. They have also used microarray technology to profile genes induced by various immunoregulatory cytokines. Dr. Powrie's group focuses on dissecting the mechanisms that govern T cell responsiveness and non-responsiveness in the intestine. Their goal is the identification of immune regulatory mechanisms (with particular emphasis on the role of T cell subsets and their cytokines), which lead to the development of tolerance to intestinal antigens, and how a breakdown in these mechanisms may lead to the development of inflammatory bowel disease. The study of Treg cells is an area in which Dr. Powrie's group has great expertise. The proposed project would utilize the strengths of both these groups to gain insights into biology of Tregs.

NIH - University of Oxford (England)

National Institutes of Health Investigator:

John Robbins

robbinsj@nichd.nih.gov

University Professor:

Richard Moxon

Molecular Characterisation of the Mechanism Underlying Lipopolysaccharide Phase Variation in Haemophilus Influenzae

The bacterium Haemophilus influenzae is a major cause of disease in children, examples being upper respiratory tract infection, septicaemia and meningitis. Lipopolysaccharide (LPS) is known to be crucial to the commensal and virulence behaviour of H. influenzae and changes in LPS structure are frequent within and between strains. Much of this heterogeneity is due to the contribution of key LPS biosynthesis genes that control the reversible expression of some LPS epitopes. These genes contain tetranucleotide repeats within the open reading frame and a change in the number of these repeats during nucleic acid replication results in translational frame shifting, altered gene expression (phase variation) and an alternative LPS phenotype. This dynamic pattern of LPS expression is thought to be crucial to the ability of this bacterium to cause disease.

In the proposed project, details of the mechanism of tetranucleotide repeat-mediated phase variation in key LPS biosynthesis genes will be investigated. The phase switching process will be analysed by constructing fusions with established reporter genes, such as ß-galactosidase, within selected LPS biosynthesis genes. The contribution of features of these genes such as utilisation of the multiple initiation codons, the length and sequence of the repeats, transcription, and the contribution of trans-acting factors will be investigated using a variety of molecular biology techniques established in the laboratory. These molecular studies on the genes will be extended to investigate LPS expression and phenotype using a range of specific monoclonal antibodies currently available. The pattern of LPS expressed will be monitored by gel electrophoresis and the detailed structure can be determined through our collaboration with several international groups.

This project will form part of a coordinated study in our laboratory and encompass our two main interests, namely the study of short nucleotide repeats and an analysis of the genetics and biology of LPS. The proposed project will allow the student to make a significant contribution to our understanding of the subject area and will be closely linked to other projects in the laboratory.

NIH - University of Oxford (England)

National Institutes of Health Investigator:

Lawrence Samelson

samelson@helix.nih.gov

University Professor:

Thomas Harder

Dynamics and Structure of Signalling Protein Complexes in the Plasma Membrane of T Lymphocytes

Triggering of the T cell antigen receptor (TCR) by engagement with a peptide-MHC ligand is the key event leading to T-lymphocyte activation. TCR-mediated downstream signals are transduced by multimolecular protein complexes, which are composed of signalling enzymes and scaffolds of transmembrane and cytoplasmic adaptors. These assemblies form in the T cell plasma membrane in close vicinity to the activated T cell receptor. Despite the vast amount of information on proteins that are involved in TCR signalling, the principles that govern the organisation, formation and disintegration of signalling assemblies in the plasma membrane are not understood. Our laboratories developed complementary, multidisciplinary approaches to study these questions.

First, we utilise biochemical analyses to probe the formation and disintegration of TCR signalling assemblies. Our experiments employ an immunoisolation procedure, in which we mechanically rip out plasma membrane fragments that contain native TCR signalling assemblies from the T cell surface. In order to assay the molecular composition of the isolates we perform Western blots, proteomics, and lipid determination (Harder and Kuhn, 2000).

Moreover, we study TCR signalling complex-formation and -disassembly using fluorescence microscopy. Different signalling proteins are expressed in T cell lines as GFP/fluorescence tagged chimeras and by video microscopy their incorporation and dissociation from signalling assemblies can be assessed with great temporal and spatial resolution (Bunnell et al., 2002). Both imaging and biochemical approaches are aided by the use of T cell lines that lack specific signalling components. Additionally, we perform biophysical and structural analyseis of TCR signalling assemblies reconstituted in vitro from recombinant proteins. These studies include calorimetric measurements of complex formation as well as structural analysis of reconstituted complexes. Finally a number of genetic models are in use to characterize the role of these signalling molecules in lymphocyte activation and development. Our long-term aim is to understand how TCR triggering is translated into proximal signalling reactions and how the dynamics of signalling assemblies regulate T cell activation.

References

Bunnell, S.C., D.I. Hong, J.R. Kardon, T. Yamazaki, C.J. McGlade, V.A. Barr, and L.E. Samelson. 2002. T cell receptor ligation induces the formation of dynamically regulated signaling assemblies. J Cell Biol. 158:1263-75.

Harder, T., and M. Kuhn. 2000. Selective accumulation of raft-associated membrane protein LAT in T cell receptor signaling assemblies. J Cell Biol. 151:199-208.

NIH - University of Oxford (England)

National Institutes of Health Investigator:

Robert Seder

rseder@mail.nih.gov

University Professor:

Adrian Hill

adrian.hill@molecular-medicine.oxford.ac.uk

Effector T Cell Vaccines for Malaria and Tuberculosis

We have described and developed a novel immunisation strategy that involves a priming immunisation with a DNA vaccine and a booster immunisation with a recombinant modified vaccinia virus Ankara (MVA) vaccine encoding the same pathogen epitopes or antigen. This regime induces high levels of CD8+ and Th1-type CD4+ T cell responses in rodents and primates. Phase I trials tob evaluate this approach in malaria commenced in 1999 and currently a programme of safety, immunogenicity and controlled challenge studies is in progress in Oxford, and field studies of these vaccines are underway in The Gambia. Phase trials of a similar approach for HIV started more recently (see entry by A McMichael) and clinical trials of a heterologous prime-boost immunisation strategy in tuberculosis commenced in 2001. Ongoing research seeks to understand the mechanisms of enhanced immunogenicity of this regime, to identify means to enhance immunogenicity further and to develop and test clinically further DNA-based vectors that will protect through effector T cells. In parallel, studies of the duration of T cell memory induced by these vaccination regimes are underway and means to enhance this memory will be evaluated.

Schneider J, Gilbert SC, Blanchard TJ, Hanke T, Robson KJ, Hannan CM, Becker M, Sinden R, Smith GL, Hill AV. Enhanced immunogenicity for CD8+ T cell induction and complete protective efficacy of malaria DNA vaccination by boosting with modified vaccinia virus Ankara. Nat Med. 1998 Apr;4(4):397-402.

Seder RA, Hill AV. Vaccines against intracellular infections requiring cellular immunity. Nature. 2000 Aug 17;406(6797):793-8.

Gilbert SC, Plebanski M, Gupta S, Morris J, Cox M, Aidoo M, Kwiatkowski D, Greenwood BM, Whittle HC, Hill AV. Association of malaria parasite population structure, HLA, and immunological antagonism. Science. 1998 Feb 20;279(5354):1173-7.

NIH - University of Oxford (England)

National Institutes of Health Investigator:

Alan Sher

asher@niaid.nih.gov

University Professor:

Siamon Gordon

The Oxford group is interested in the immunobiology of antigen presenting cells, especially macrophages. These cells express a range of surface receptors that recognise molecular structures (patterns) displayed by pathogens - see for example recent discovery of a new b glucan receptor (Nature 413: 36-37 - Brown & Gordon). It is not known how antigens from parasites such as Schistosome egg antigens (SEA) and other sources induce a Th2 rather than a Th1 response. We postulate the existence of APC receptors for SEA and wish to apply expression cloning to identify such a receptor on APC. Dr. Sher's group has complementary expertise in parasitic infection and the link between innate and acquired immune systems.

Also see Gordon lab web site:
http://dunn1.path.ox.ac.uk/~cholt/

NIH - University of Oxford (England)

National Institutes of Health Investigator:

Richard Siegel

rsiegel@nih.gov

University Professor:

Gavin Screaton

screaton@molbiol.ox.ac.uk

Structure-Function studies of Death Receptor Signaling

The Fas receptor, also known as APO-1 or CD95, is a key initiator of apoptotic programmed cell death in a variety of cell types. CD4+ T cells are unique in their ability to commit ‘suicide’ by stimulating their own Fas receptors with secreted or membrane-bound Fas ligand. These interactions lead to downmodulation of autoimmune T and B cell responses critical to maintaining immunological self-tolerance. Recent findings have suggested that Fas exists as a preformed oligomer before FasL binding, explaining the effect of a number of pathogenic mutations in Fas, and suggesting novel therapeutic targets for intervention in this immunoregulatory signaling pathway. In a joint project with Drs. Gavin Screaton (Oxford) and Richard Siegel (NIAMS, NIH), Adrian Lobito is studying the structure and function of Fas/Fas Ligand interactions. He is producing large amounts of biologically active Fas Ligand and soluble receptors for detailed structural analysis, and testing in functional assays. These approaches will lead to a more complete understanding of the structural basis of Fas signaling, as well as explore novel approaches to manipulating Fas signaling to ameliorate autoimmune diseases.

NIH - University of Oxford (England)

National Institutes of Health Investigator:

Ronald Summers

rms@nih.gov

University Professor:

MIchael Brady

jmb@robots.ox.ac.uk

Computer-Aided Detection to Identify Early Colon Cancer Before It Spreads

Colon cancer is the second leading cause of cancer death in the United States and Europe. Current research suggests that early detection of colon cancer can lead to improved survival. CT colonography ("virtual colonoscopy") is a radiologic test that can detect colon cancer in its earliest manifestations.

Two important limitations of CT colonography (CTC) are that it is time consuming for radiologists to interpret and perceptual errors, leading to misdiagnoses, are common. To address these limitations, we are developing computer-aided detection methods to help radiologists find abnormalities on the scans. These methods include novel algorithms from the fields of computer vision, differential geometry, computer graphics, deformable models, statistical pattern recognition, and neural networks. Clinical validation and development of large databases of CTC images are also major focus areas.

The NIH Computer-Aided Diagnosis Laboratory has state of the art computing facilities and is located on the vibrant NIH campus in Bethesda, Maryland, USA. Graduate students are encouraged to publish and participate in weekly lab meetings and journal club.

The Medical Vision Laboratory (MVL) in the Department of Engineering Science at Oxford was founded in 1994. The MVL's research thrusts and expertise include: x-ray mammography and CT, MRI (normal, contrast-enhanced, functional, and MRA), and ultrasound (of the heart and breast). Image fusion is also a major interest.

Interested applicants are encouraged to contact Dr. Ronald Summers (rms@nih.gov, http://www.cc.nih.gov/drd/summers.html) or Professor Michael Brady (jmb@robots.ox.ac.uk, http://www.robots.ox.ac.uk/~jmb) for further information.

NIH - University of Oxford (England)

National Institutes of Health Investigator:

Peter Sun

psun@niaid.nih.gov

University Professor:

George Gao

The collaboration between the laboratories of Peter Sun and George Gao focuses on the structure and function of NK receptors, their ligand recognition and the balance between the inhibitory and non-inhibitory NK receptors that determines the protection versus killing by the innate immunity. One family of such inhibitory receptors consists of members of CD94/NKG2 receptors that recognize non-classical class I HLA-E. Most natural killer cells and a subset of T cells express CD94/NKG2 receptors and their recognition of HLA-E presumably protects healthy cells from NK mediated killing. At the structural level, Dr. Sun's group plans to determine the structure of CD94/NKG2 in complex with HLA-E using X-ray crystallography. Our goal is to delineate the molecular mechanism of this receptor-HLA-E recognition. At the functional level, Dr. Gao's group will study the balance between the inhibitory CD94/NKG2 receptor and other non-inhibitory receptors, such as NKG2D, through mutational analysis using a cell based killing assay and protein-protein interaction assays (e.g. BiaCore Binding analysis). A graduate student, interested in structural immunology, will study the function of these receptors in Dr. Gao's lab as well as the structure of the receptor-HLA-E complex in Dr. Sun's lab.

NIH - University of Oxford (England)

National Institutes of Health Investigator:

Masaki Terabe

mt180u@nih.gov

University Professor:

Vincenzo Cerundolo

vincenzo.cerundolo@imm.ox.ac.uk

The overall project deals with the role of NKT cells, restricted by CD1d, by using CD1d-tetramer developed by Cerundolo's laboratory, as a part of the innate immune system that interfaces with and in part regulates the adaptive immune system of T and B lymphocytes in health and disease. We are especially interested in the role of these cells in cancer and chronic viral infection such as HIV and hepatitis C virus infection.

The immune system has two arms to defend against pathogens or abnormal cells in the body. One is innate immunity and the other is adaptive immunity. Understanding the interface between innate and adaptive (acquired) immunity is of great current interest to the field of immunology because it is central to understanding the mechanisms of regulation of immune responses. Natural Killer T (NKT) cells are T cells which also express NK cell markers, and are usually restricted by the non-classical class I MHC molecule CD1d, presenting a glycolipid rather than a peptide. This cell has some features of both innate and adaptive immune cells, and recently has been shown to regulate many immune reactions which cause and prevent disease. However, since this is a very small population, it has been very difficult to study. Tetramer technology is a one of the cutting edge technologies permitting detection of a small number of T cells by using a flow cytometer. The Cerundolo laboratory at Oxford University has developed tools to identify these NKT cells and to study their interaction with the adaptive immune system of CD4+ and CD8+ T cells as well as B cells. The Oxford laboratory has also developed protocols to load CD1d tetramers with single glycolipids to identify physiologically relevant ligands recognized by these NKT cells. The Berzofsky/Terabe laboratory at NIH has discovered a novel negative regulatory pathway initiated by CD1d-restricted NKT cells that downregulates tumor immunosurveillance. The pathway involves secretion of IL-13 by the NKT cells that induces a CD11b+Gr-1+ myeloid cell to make TGF-beta, which then suppresses the induction of the CD8+ cytotoxic T lymphocytes (CTL) that mediate the tumor immunosurveillance. The cell controlling this regulatory pathway is the NKT cell, but the signals that activate it and the factors that determine whether this cell makes IL-13 and acts as a suppressor or makes interferon-gamma and facilitates the anti-tumor response are unknown. Thus, it would be of great interest to isolate the relevant glycolipids produced by the tumor cell that bind to CD1d and activate this regulatory NKT cell. The approaches developed by the Oxford component of the collaboration should make it possible to identify such CD1d ligands and determine their role in initiating the regulatory response.

Furthermore, it is important to define the role of these negative regulatory NKT cells in human cancer and chronic viral infections in which immunodeficiency is observed, such as HIV infection. The ability to stain these cells with tetramers of human CD1d loaded with different lipids, and to further characterize these cells by the limited repertoire of T cell receptors used, should facilitate the exploration of the role of these innate immune cells in regulating adaptive immune responses in human disease.

The student would work on CD1d molecules, tetramers, their lipid ligands, and their ability to prime a CD1d-restricted immune response in Oxford, on the murine cancer models at NIH, and on human NKT cells in different disease states in both Oxford and NIH.

NIH - University of Oxford (England)

National Institutes of Health Investigator:

Richard Veech

rveech@mail.nih.gov

University Professor:

Kieran Clarke

Kieran.Clarke@bioch.ox.ac.uk

Role of Homocysteine in Cardiovascular and Cerebrovascular Diseases

Elevated plasma homocysteine concentrations are associated with increased risk of cardiovascular and cerebrovascular diseases,1,2 including Alzheimer's disease,3 but it remains to be determined whether homocysteine is a causal factor or a marker for another risk factor. Because homocysteine is generated intracellularly and can accumulate in cells, it may be that homocysteine can directly modulate the activity of both large (enzymes, receptors) and small molecules (nitric oxide, glutathione).4,5 The first aim of this project is to determine whether, and the cellular mechanism whereby, increased homocysteine has adverse effects on heart and brain. Elevation of plasma homocysteine can result from deficiency of vitamins B6 and B12 or folic acid and levels can be lowered by supplementation with high doses of folic acid, which is required for efficient remethylation of homocysteine to methionine. However, for reasons unknown, changes in plasma homocysteine in response to folic acid supplementation are highly variable.6 Consequently, the second aim of this project is to determine the cellular mechanism underlying the variable response to folic acid. Heart and brain will be studied in rat and mouse models of hyperhomocysteinemia, using magnetic resonance imaging and spectroscopy plus other techniques. This study will reveal whether homocysteine is involved directly in heart and brain diseases, and may have important clinical implications for therapeutic strategies.

References

1. Duan W, Ladenheim B, Cutler RG, Kruman II, Cadet JL, Mattson MP. Dietary folate deficiency and elevated homocysteine levels endanger dopaminergic neurons in models of Parkinson's disease. J Neurochem 80:101-110, 2002.
2. Vollset SE, Refsum H, Tverdal A, Nygard O, Nordrehaug JE, Tell GS, Ueland PM. Plasma total homocysteine and cardiovascular and noncardiovascular mortality: the Hordaland Homocysteine Study. Am J Clin Nutr 74:130-136, 2001.
3. Seshadri S, Beiser A, Selhub J, Jacques PF, Rosenberg IH, D'Agostino RB, Wilson PWF, Wolf PA. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. New Engl J Med 346:476-483, 2002.
4. Werstuck GH, Lentz SR, Dayal S, Hossain GS, Sood SK, Shi YY, Zhou J, Maeda N, Krisans SK, Malinow MR, Austin RC. Homocysteine-induced endoplasmic reticulum stress causes dysregulation of the cholesterol and triglycseride biosynthetic pathways. J Clin Invest 107:1263-1273, 2001.
5. Kruman II, Culmsee C, Chan SL, Kruman Y, Guo Z, Penix L, Mattson MP. Homocysteine elicits a DNA damage response in neurons that promotes apoptosis and hypersensitivity to excitotoxicity. J Neurosci 20:6920-6926, 2000.
6. Malinow MR, Duell PB, Williams MA, Kruger WD, Evans AA, Anderson PH, Block PC, Hess DL, Upson BM, Graf EE, Irvin-Jones A, Wang L. Short-term folic acid supplementation induces variable and paradoxical changes in plasma homocysteine concentrations. Lipids 36:S27-S32, 2001.

NIH - University of Oxford (England)

National Institutes of Health Investigator:

Daniel Weinberger

weinberd@intra.nimh.nih.gov

University Professor:

Paul Harrison

paul.harrison@psych.ox.ac.uk

Our research focuses on the core neurobiology of schizophrenia, with particular emphasis on genetic contributions to neuropathological, neurophysiological and neuropsychological endophenotypes. Methods used include functional and structural brain imaging, neuropathology, molecular biology, candidate gene studies, and animal models. We already have active collaborations between our labs, which you will cement and enhance.

The specific project in question will investigate one of the recently discovered susceptibility genes for schizophrenia, the mGluR3 metabotropic glutamate receptor (GRM3). The GRM3 gene is involved, along with a cysteine-glutamate transporter in regulation of synaptic glutamate. This is of interest in schizophrenia since much work from our labs and others has implicated glutamatergic dysfunction as being important in the pathophysiology of the disorder. The project will examine several aspects of GRM3, including detailed gene expression analyses in human brain, mice bearing human GRM3 transgenes, in vivo correlates of mGluR3 polymorphisms, etc…This project will result in the student acquiring a broad range of training in molecular analyses of human and animal tissue and in vivo techniques in humans (e.g. functional neuroimaging) and in animals related to genetic mechanisms of susceptibility for psychosis. The in vitro brain studies would mainly be based in Oxford, with the in vivo and transgenic work being based at NIMH?

Recent references illustrating our work and interests:

Weinberger DR et al (2001) Prefrontal neurons and the genetics of schizophrenia. Biological Psychiatry 50: 825-844

Egan et al (2003) The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell 112: 257-269

Harrison PJ (1999) The neuropathology of schizophrenia. Brain 122: 593-624.

Harrison PJ, Owen MJ (2003) Genes for schizophrenia? Recent findings and their pathophysiological implications. Lancet 361: 417-419

NIH - University of Oxford (England)

National Institutes of Health Investigator:

Thomas Wellems

tew@helix.nih.gov

University Professor:

Dominic Kwiatkowski

dominic@well.ox.ac.uk

Malaria Protection by Hemoglobin Mutations

Human erythrocytes possess common genetic variants which appear to have evolved because of their protective effect against Plasmodium falciparum malaria. In West Africa there are two prevalent mutations of the HBB gene, hemoglobin S (ß6 GluàVal) and hemoglobin C (ß6 GluàLys), whose frequencies vary widely across the region. Epidemiological studies of the Dogon in Mali1 and the Mossi in Burkina Faso2 indicate that the dominant protective effect is due to HbC, whereas studies of the Dagomba in Ghana3, the Hausa and Fulani in Nigeria4, and the Mandinka in The Gambia5 indicate that HbS is a more important determinant of protection in these populations. These findings suggest that the effects of HbC and HbS on antimalarial immunity may vary among West African populations, possibly due to ethnic factors such as other disease-modifying genetic polymorphisms, or environmental factors that affect parasite phenotype or the pattern of disease transmission. We are proposing a collaborative project involving scientific colleagues in West Africa6 as well as the NIH and Oxford labs. The goal will be to combine epidemiological and immunological analyses with dense genetic mapping of the HBB locus and other candidate genes, in order to explore the molecular basis of protection by the hemoglobinopathies.

1. Agarwal, A. et al. Blood 96, 2358-2363 (2000).
2. Modiano, D. et al. Nature 414, 305-308 (2001).
3. Edington, G.M. and Liang, W.N. Brit.Med.J. 2, 143-145 (1957).
4. Molineaux, L., & Gramiccia, G. The Garki Project. Research on the Epidemiology and Control of Malaria in the Sudan Savanna of West Africa (World Health Organization, Geneva, 1980).
5. Hill, A.V.S. et al. Nature 352, 595-600 (1991).
6. Science 290, 431-433 (2000).

NIH - University of Oxford (England)

National Institutes of Health Investigator:

Kenneth Yamada

kenneth.yamada@nih.gov

University Professor:

Helen Mardon

skennedy@molbiol.ox.ac.uk

Fibronectin-Integrin Interactions

Fibronectin is a large, modular extracellular matrix glycoprotein with functions in diverse biological processes such as adhesion, proliferation, differentiation and invasion. Thus fibronectin has a central role in normal tissue development and homeostasis and has been implicated in the pathogenesis of many diseases, including cancer. Its function is mediated by the interaction of specific fibronectin structural domains with the integrin family of transmembrane heterodimeric receptors. Binding of the fibronectin ligand to different integrins triggers bidirectional signalling responses that lead to profound modulation of cell behaviour.

Fibronectin can bind to a number of different integrin heterodimers, including avb3 and a5b1. The major integrin binding site is the RGD-containing amino acid motif that resides in the tenth FIII domain. However additional binding sites exist in adjacent domains that confer synergistic activity to RGD. The crystal structure of the integrin avb3 in complex with an RGD peptide has given new insight into the molecular mechanisms of integrin-fibronectin interactions (Xiong st al., Science 2002, 296:151-5), but the molecular basis of integrin binding specificity is not clear. The aim of this project is to determine the structural basis and functional consequences of the interaction of fibronectin FIII domains with different integrin receptors. The project will involve the application of a combination of approaches involving structural and functional analyses with the use of cell and molecular methodologies. The understanding of the molecular architecture of specific integrin-ligand interactions and the functional consequences of these interactions will contribute towards the design of new integrin agonists and antagonists and will thus have potential clinical application in a wide range of diseases.

The research is part of collaborative programme which will facilitate interaction with other research groups within the Division of Medical and Biological Sciences. The project will appeal to students who wish to be exposed to multi-disciplinary research, and there will be scope for the student to experience a broad range of cell and molecular approaches and to then develop aspects of the project according to his/her interests.

NIH - University of Oxford (England)

National Institutes of Health Investigator:

Jonathan Yewdell

jyewdell@niaid.nih.gov

University Professor:

Andrew McMichael

andrew.mcmichael@ndm.ox.ac.uk

Immunodominance and Optimisation of Vaccines

The CD8 +T cell response to viruses tends to focus on a small number of determinants, sometimes only one, even though the virus may encode more than 50,000 amino acids. This phenomenon is known as immunodominance, and it poses serious problems for vaccines. Andrew McMichael's laboratory in Oxford is designing vaccines to stimulate strong CD8+ T cell responses to HIV. The approach is to immunize first with DNA encoding an HIV gene and then to boost with a recombinant modified vaccinia virus Ankara expressing the same gene. Early trials in humans show that this approach is immunogenic and stimulates CD8+ T cell responses. It will be crucial to make responses to as many determinants as possible; both to cover the diversity of HIV strains circulating in the human population and to prevent the emergence of determinant-escape variants selected by highly restricted responses. The conventional approach is to link a series of HIV proteins into one DNA construct, but this unlikely to avoid immunodoiminance. Therefore, McMichael's laboratory will design constructs each encoding one protein and immunize with several constructs at once. This project will study these responses in mice that are transgenic for the HLA molecules A2 or B27. The immune responses will be dissected to determine what factors influence the breadth of the T cell response made. The dissection will be carried out in collaboration with Jonathan Yewdell and Jack Bennink at NIH who have performed seminal work in the study of immunodominance.

Web links:
http://www.jr2.ox.ac.uk/ndm/groups/immunology.htm#groups
http://www.niaid.nih.gov/dir/labs/lvd/bennink.htm

NIH - University of Oxford (England)

National Institutes of Health Investigator:

Jonathan Yewdell

jyewdell@niaid.nih.gov

University Professor:

Vincenzo Cerundolo

vincenzo.cerundolo@imm.ox.ac.uk

Immunodominance During Vaccine Induced CTL Responses

Recombinant vaccines encoding strings of virus or tumor derived peptides and/or proteins are currently being designed for use against both cancer and infectious diseases. These vaccines aim to induce cytotoxic immune responses against several antigens simultaneously. We developed a novel tetramer-based technique to directly monitor the CTL response to such vaccines in HLA-A2 transgenic mice. We found that priming and boosting with the same poly-epitope construct induced immune responses that were dominated by CTL of a single specificity. When a mixture of viruses encoding single proteins was used to boost the poly-epitope primed response, CTL of multiple specificities were simultaneously expanded to highly effective levels in vivo. In addition, we show that a pre-existing response to one of the epitopes encoded within a poly-epitope construct significantly impaired the ability of the vaccine to expand CTL of other specificities.

It is out intention to characterize the immunodominance of vaccine encoding poly-epitope and poly-protein constructs. Experiments will be carried out in vitro, using purified proteasomes, and in vivo using A2 transgenic mice. Particular attention will be focused on the use in vivo of vital microscopy to identify the population of virus infected antigen presenting cells responsible for the priming of naïve CD8+ cells and boosting of antigen experienced cytotoxic T lymphocytes.